ENDOCRINE AND REPRODUCTIVE DEVELOPMENT OF THE HOLSTEIN BULL FROM BIRTH THROUGH PUBERTY

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KEITH LINDSAY MACMILLAN
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ABSTRACT

ENDOCRINE AND REPRODUCTIVE DEVELOPMENT OF THE HOLSTEIN BULL FROM BIRTH THROUGH PUBERTY

by Keith L. Macmillan

A total of 65 Holstein bulls were killed in groups of five at monthly intervals from birth to 12 months of age. The pituitary levels of lutenizing hormone (LH), follicle stimulating hormone (FSH) and growth hormone (GH), hypothalamic levels of luteinizing hormone-releasing factor (LH-RF) and blood plasma levels of LH were measured and compared to changes associated with the development of the reproductive tract.

The concentrations of pituitary and plasma LH were measured by ovarian ascorbic acid depletion assays. The potency of pituitary LH at birth was 0.76 ug NIH-LH-B3 equivalents per mg fresh pituitary. At 1 month of age pituitary LH potency was 4.88 ug LH per mg and thereafter there was an irregular decline to 1.96 ug LH per mg at 12 months of age. In contrast, the pituitary content of LH was greatest at 6 months of age (2.83 mg NIH-LH-B3 equivalents per anterior pituitary gland). The changes in both pituitary LH potency and content may have been associated with reproductive development but the limits

of puberty could not be defined by any dramatic changes in either LH parameter.

The plasma LH was concentrated using an acetone extraction procedure and injected at dose levels equivalent to 100 or 25 ml plasma per assay rat. Extration efficiency was 25 percent. Vasopressin contamination did not modify the results. The amount of LH in the blood plasma did not change from birth to 2 months, increased to 4 months and increased again between 6 and 10 months of age. The estimated amounts of pituitary LH released per animal per day were 52, 208, 399 and 610 ug NIH-LH-B3 equivalents at 2, 4, 8 and 10 months of age, respectively. Although LH-RF activity, measured by ovarian ascorbic acid depletion assays, could not be detected in hypothalami obtained from bulls of 4 months of age or less, the increase in plasma LH between 6 and 10 months of age was associated with an increase in levels of hypothalamic LH-RF. As a safeguard against measuring LH instead of LH-RF, all of the extracted LH-RF samples were boiled for 10 min and dialyzed. The dialysate was lyophylized and injected at dose levels equivalent to 0.50 or 0.25 hypothalami per assay rat. Similarly treated cerebral cortical tissue did not depress ovarian ascorbic acid concentration and the preparative procedures inactivated over 99 percent of added NIH-LH-B2 LH.

The age-trends in the weight and DNA and RNA contents of the paired seminal vesicles were very similar to

the changes in plasma LH. However, the greatest increases in the contents of fructose and citric acid occurred from 6 months of age. The relationships indicated that increases in levels of plasma LH produced increases in testicular androgen synthesis which in turn stimulated seminal vesicular growth and secretory activity.

The pituitary potency of FSH, measured by the ovarian weight augmentation assay, was greatest at 2 months of age (0.24 ug NIH-FSH-S3 equivalents per mg fresh pituitary) and then declined irregularly to 11 months of age (0.07 ug per mg). The pituitary content of FSH, like LH, was greatest at 5 and 6 months of age. The pituitary potency of GH, measured by the tibial response in hypophysectomized rats, increased from 16.09 to 126.69 ug NIH-GH-B9 equivalents per mg fresh anterior pituitary at 1 and 4 months of age, respectively, and then declined to 24 ug equivalents per mg at 12 months of age.

It appeared that the most rapid period of reproductive development of the Holstein bull commenced at 2 months of age. To 6 months of age, the development was due to FSH stimulation in conjunction with low levels of plasma LH. From 6 to 9 months of age reproductive development was rapid and was due to increasing levels of plasma LH. Changes in the reproductive tract after 9 months of age were quantitative rather than qualitative. An exception to this generalization was that a sexually mature rate of sperm production of 52.83 million sperm per

gram testicular parenchyma was not attained until 11 months of age, although spermatids were detected in the testes of one bull as early as 5 months of age and in all bulls by 8 months of age.

ENDOCRINE AND REPRODUCTIVE DEVELOPMENT OF THE HOLSTEIN BULL FROM BIRTH THROUGH PUBERTY

By

Keith Lindsay Macmillan

A THESIS

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"We judge ourselves by what we feel capable of doing, while others judge us by what we have already done."

--Henry Wadsworth Longfellow

BIOGRAPHICAL SKETCH

of

Keith Lindsay Macmillan

Born in Sydney, Australia, on April 27, 1940, Keith Lindsay was almost named Jock. This latter name stuck and is used almost exclusively by those who know him. His high school education was completed at Albury Grammar School, Albury, N. S. W. He obtained his matriculation certificate in 1957 with honours in Chemistry, A passes in Physics, Economics, English and Mathematics II and a B pass in Mathematics I. In light of these results he was awarded a Commonwealth Scholarship, an Australian Primary Producers' Board Scholarship and a Victorian Department of Agriculture Cadetship. He also won the R. S. L. Prize which is awarded annually to the student who is the son of an ex-serviceman and who obtains the best results in the matriculation exam from among eligible students in the Albury District.

Acceptance of the Cadetship allowed Jock to attend Massey Agricultural College, Palmerston North, New Zealand. He graduated B. Agr. Sc. from this institute in April, 1962. He obtained his M. Agr. Sc. with Second Class Honours in Animal Science from the same institute in April, 1963. His thesis, entitled "The Use of Oestrous Cows for

the Pre-Collection Preparation of Mature Bulls Standing at an Artificial Breeding Centre" was the result of research conducted with the New Zealand Dairy Production and Marketing Board.

From February, 1963, to August, 1964, he was employed by the Dairy Division of the Victorian Department of Agriculture as an Assistant Dairy Husbandry Officer. His duties included lecturing Physics and Chemistry to students at the School of Dairy Technology, Werribee, and advising dairy farmers on problems related to dairy cattle nutrition.

In September, 1964, he was awarded a graduate research assistantship by the Department of Dairy, Michigan State University. This position allowed him to study for his Ph.D., which he completed in April, 1967.

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The possibility of my studying for a Ph.D. at a university like Michigan State was primarily due to the generosity of the Dairy Department who made the necessary funds available. However, the success of the studying and the research, and my development as an individual capable of conducting imaginative investigations in a systematic manner is largely the result of the guidance and encouragement provided by Dr. Harold Hafs. While I have a personal feeling of indebtedness, I hope my future productivity will serve as a source of satisfaction for my mentor.

I owe specific thanks to Dr. Allen Tucker for his guidance during the year that Dr. Hafs was at Harvard, and for his interest, advice, and assistance throughout my two and one-half years at East Lansing. I am also grateful of the cooperative assistance profferred by Drs. Anderson, Reineke, Thomas and Emery.

The large amount of work necessary to produce the data in this thesis is the result of a team effort. Key members in this team were Mrs. Helga Hulkonen and Miss Susan Jeffries. Their cooperation and conscientious work is gratefully appreciated. Similarly, I am indebted to

my student contemporaries for their interest, stimulation and assistance. Members of this august group included Claude Desjardins, Kenneth Kirton, Max Paape, Yogi Sinha, Art Hackett and Robert Wetteman.

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INTRODUCTION

During the last 20 years the number of cows which are inseminated artificially has increased dramatically. This increase has been associated with a greater demand for semen from superior sires so that the genetic advantages accompanying the use of artificial insemination can be more fully realized. In turn, this demand has stimulated research to extend the use of superior sires either by improvements in semen preservation, or by increasing the number of sperm harvested from each bull. This latter approach has resulted in a large number of publications concerning the reproductive functions of the bull. However, the great majority of these studies have been on the mature sire.

The advent of sire-proving programs in artificial breeding organizations has increased the need for basic information on the reproductive development of the young bull. The generation interval in dairy cattle is comparatively large among domestic animals and consequently, a prospective sire should be evaluated at the earliest possible age if he is to be fully utilized or wisely discarded. Thus, the age at which fertile sperm can be obtained from a young bull is of major economic importance. If this age could be reduced, or if the sperm output of

the young bull could be increased, considerable benefit would be realized. Unfortunately, little is known of the endocrinological changes associated with the normal pattern of reproductive development in the bull. To date, the few studies which have been conducted with bulls have been based largely on macro- or micro-morphological changes associated with the sexual development of the reproductive tract. These parameters were used as indirect estimates of the changes in the blood levels of testosterone and changes in this hormone were considered to reflect changes in the blood titers and the pituitary potencies of the gonadotropins. Studies in laboratory animals have suggested that the onset of sexual development in the young animal appears to be associated with changes in the hypothalamus which mediates the functions of the anterior pituitary gland. To the author's knowledge, the hypothalamo-hypophyseal relationship has not been studied in the prepuberal bull. The measurement of changes in target organs which may not be directly dependent upon the pituitary is a rather devious method for determining the specific alterations in the hypothalamo-hypophysial system which may result in the reproductive development associated with puberty.

The present study was primarily initiated to obtain data on the changes in the levels of pituitary gonadotropins which normally occur during the sexual development

of the bull. The study was also designed to determine whether such changes were associated with changes in the hypothalamus, the blood levels of luteinizing hormone and the structure and biochemical composition of the testis, which is the direct target organ, and the accessory reproductive glands.

A concomitant objective was to obtain basic data on the gonadotropic changes associated with puberty in an animal which has a distinct pre-pubic period, followed by a five-to-six month period of sexual development and then the attainment of sexual function all within the first year of life. These epochs are not so clearly definable in laboratory animals such as the rat, because of the rapidity of the changes. Although the end of puberty in the male is not heralded by the onset of estrous cycles as it is in the female, an advantage in studying this topic in the male is that the role of the male's pituitary is not complicated by the cyclic gonadotropic fluctuations which typify the estrous cycle. Finally, this study would complement the results from a similar study conducted by Desjardins (1966) in heifers. To date, the only systematic comparisons of the changes in the gonadotropins of the two sexes have been in the rat, and any differences between the reported data for rats and those demonstrated by this research could have considerable import on subsequent studies involving the changes associated with puberty.

REVIEW OF LITERATURE

A. THE DEFINITION OF PUBERTY AND FACTORS AFFECTING SEXUAL DEVELOPMENT

At birth, the bovine testis is composed of small solid sex chords which are widely separated from each other. By one year of age, the diameter of the seminiferous tubules is essentially that found in a mature bull, spermatogenesis is apparent, and the young bull is capable of paternity (Abdel-Raouf, 1960, and Baker et al., 1955). Thus, between birth and one year of age, the dairy bull experiences the dramatic changes associated with sexual development. This process includes an ill-defined phase called "puberty." Several definitions of puberty may be found in the literature although the concept of the meaning of this term varies considerably. Donovan and van der Werff ten Bosch (1965) defined puberty as "the phase of bodily development during which the gonads secrete hormones in amounts sufficient to cause accelerated growth of the genital organs and the appearance of secondary sexual characters." This definition has been adopted in the interpretation of the literature and of the data obtained from the bulls used in this study.

Puberty is usually recognized by the appearance of outward signs attributable to the action of the sex hormones. In many studies in the female rat, the criterion

used has been vaginal opening which immediately precedes the onset of estrous cycles. However, the definition of puberty used in this thesis would interpret vaginal opening as the termination of puberty. In the male, the criteria for determining the termination of puberty are not as precipitous or as dramatic. Webster and Young (1951) and Bond (1945) reported that copulatory activity was apparent in male guinea-pigs and hamsters well before the animals became fertile. Data obtained by Bratton et al., (1959) showed that some bulls on normal levels of nutrition would ejaculate at 38 weeks of age. By contrast, other bulls which appeared capable of producing semen, did not appear to have sufficient libido to mount and ejaculate. Therefore, behavioral criteria may not be good indicators of the attainment or termination of puberty in the male. A better criterion may be the presence of sperm in the excurrent ducts. Once sperm have arrived in the cauda epididymidis and the proximal ductus deferentia, they can probably be obtained by ejaculation or electro-ejaculation (Wolf et al., 1965). Thus, detection of sperm in both these regions would mean that puberty had ended. However both a bull's sperm output and libido are known to increase considerably after puberty.

Abdel-Raouf (1960) concluded that in the Swedish Red-and-White breed, the testes complete qualitative development by 40 weeks of age. The accessory glands

attain comparable stages of development 5 to 8 weeks earlier. Thus, he considered that puberty had ended by 40 weeks of age in well fed bulls. Bratton et al., (1959) reported the average age of the onset of semen production, as determined by ejaculation into an artificial vagina, was 43 weeks of age in Holstein bulls fed on a medium level of nutrition (100 percent Morrisson's allowance). However, Baker et al., (1955) found that there was considerable variation within a breed in the age at first mounting and the age at which the first ejaculate was obtained. These variations may have been of genetic origin if the variation within a breed is similar to variations between breeds. Salisbury and VanDemark (1961) noted that Brown Swiss bulls are usually 2 to 3 months later than most other dairy breeds in their sexual development.

Genetic factors are not the only contributants to variations in the age of puberty. The effect of varying the plane of nutrition on the reproductive development of the bull has been extensively studied. Bratton et al., (1959) used three experimental feeding levels. The average ages for the onset of semen production were 37, 43 and 51 weeks for the bulls receiving high, medium or low levels of nutrition. Abdel-Raouf (1960) found that a low plane of nutrition delayed puberty by 8 weeks. These variations in the plane of nutrition do not affect subsequent fertility (Bratton et al., 1960). The Cornell study also

showed that ad libitum feeding of high quality feeds was the most expensive method but allowed young sires to be proved 4 months earlier than a bull fed on a normal level of nutrition.

Other environmental factors which have been reported to influence the age of puberty and reproductive function, include climate and temperature, light and season, and stress. All of the studies on the influence of environment on the age of puberty have involved laboratory animals or humans (see review by Donovan and van der Werff ten Bosch, 1965, pp. 26-30). Relative to environmental factors on reproductive function in bulls, Amann et al., (1966) showed that Holstein bulls exhibit significant monthly fluctuations in sperm output and in other seminal components. Mercier and Salisbury (1947) reported that the fertility of mature bulls was lowest in winter, improved in spring and reached a peak in summer and fall. In contrast, the fertility of young bulls, which were less than 4 years old, declined in summer and improved in fall. They concluded that older bulls were probably more tolerant to the effect of the high temperatures than were younger bulls. This hypothesis is supported by the results of Casady et al., (1953) who showed that young bulls which were continuously subjected to a temperature of 85° F. for 5 weeks or longer exhibited impaired spermatogenesis.

Because no reports were found which specifically considered the influence of environmental factors on the period of puberty in bulls, perhaps the best that can be done is to accept the conclusion for other species (Donovan and van der Werff ten Bosch, 1965); "that both extreme heat and extreme cold delay the time of onset of puberty."

Usually bulls and heifers are separated at 4 to 5 months of age. Whether such segregation influences the time of puberty has not been established. Morton et al., (1963) have reported that in rats, females reared with males reach puberty earlier than those separated on the basis of sex. They also found that mild stress in early infancy caused earlier vaginal opening and earlier onset of estrus in females. In male rats, it caused increased seminal vesicular and prostatic weights at 35 days of age.

B. CHANGES IN THE HORMONES OF THE ANTERIOR PITUITARY WITH ADVANCING AGE

The onset of puberty has long been associated with changes in the gonadotropin content of the pituitary, and more recently with a maturation or alteration of the role of the hypothalamus. The earlier work by Clark (1935), McQueen-Williams (1935) and Lauson et al., (1939) involved the measurement of total gonadotropins (TG) as distinct from the specific measurement of luteinizing

hormone (LH) or follicle stimulating hormone (FSH). In the female rat, pituitary TG potency increases gradually during the first 2 weeks of life, rapidly during the third week and then declines just prior to the onset of puberty. The first two writers also showed that the peak TG potency in male rats was 1 week later than in the female rat, but then also declined. Hoogstra and Paesi (1955) reported that the total FSH content of the immature female rat pituitary was greater than that of the adult female or immature male pituitary, but was not as great as that of the adult male pituitary. The potency of FSH in adult male rat pituitaries appeared to be five times greater than that found in pituitaries from females of comparable age. The LH potency was also greatest in mature male rats and differences between the sexes in prepubertal rats were small. Later work by Ramirez and McCann (1963) suggested that in fact the immature female pituitary contains more than twice the amount of LH as the immature male pituitary. They could find little difference in the LH content of the pituitaries from immature and mature female rats.

Wolfe and Cleveland (1931) tested the capacity of anterior pituitary tissue, taken from mature and immature female rabbits, to induce ovulation in sexually mature rabbits. They concluded that the pituitary from immature female rabbits as young as 3 months of age, and from mature females, had equal capacity to induce ovulation.

However, Hill (1934) found that the mature female rabbit pituitary was the most potent and that male pituitaries and pituitaries from 3 to 4 month old females were of similar potency. Hill used the same assay as Wolfe and Cleveland and also found that in cats and rats the mature male pituitary was more potent than the female pituitary but that in guinea pigs, rabbits and dogs the female pituitary was more potent.

Desjardins (1966) found that the average potency of LH in Holstein heifer pituitaries increased four-fold from birth to 3 months of age and maintained this potency to 7 months of age, when there was a linear decline from 10.3 ug LH per mg of anterior pituitary tissue to 4.8 ug per mg at 1 year of age. Pituitary FSH potencies in the same heifers revealed a significant peak at 1 month of age and a slight decline in potency following puberty. In contrast, Magistris (1932) found a lower level of FSH in the hypophyses of calves than in cows. Bates et al., (1935) found essentially similar concentrations of FSH in pituitaries obtained from fetuses, calves and cows although the calves were slightly higher than cows. Also, bulls and cows did not differ appreciably in FSH potency in that study.

The gonadotropins are not the only anterior pituitary hormones to show potency changes with advancing age. In cattle, Reece and Turner (1937) found that pituitary

prolactin potency appeared to increase slowly from birth to 1 year of age in both sexes. Thereafter, bulls showed only minor changes, but the potency in heifers continued to increase and showed a dramatic increase at the onset of lactation. Other mammals exhibit different patterns in potency changes. For example, the pituitary prolactin potency of the male guinea pig shows an increase which is parallel to body weight from 170 to 800 g but prolactin potency does not change in male rats between 80 and 340 g body weight. The male rabbit is at the other extreme as the prolactin potency in the immature male rabbit is three times greater than that of the mature male rabbit's pituitary (Reece and Turner, 1937). Until the role of prolactin in the reproductive function of the male is more clearly defined, the significance of these species variations may not become apparent.

Armstrong and Hansel (1956) found that the concentration of growth hormone in the anterior lobe of heifer pituitaries showed little change until 32 weeks of age and then gradually declined from 11.66 mg per gram of wet anterior lobe tissue to 7.73 mg per gram at 80 weeks of age. In female rats, Solomon and Greep (1958) reported little variation in growth hormone potency between the ages of 10 and 630 days.

Cattle show little variation in the pituitary content of thyrotropic hormone (TSH). Reece and Turner (1937) found that the TSH potency increased slightly in both

sexes from birth to 10 months of age and then declined slowly. Their results with heifers were in agreement with those reported by Armstrong and Hansel (1956). In rats, Turner and Cupps (1939) found a marked increase in the TSH potencies of rat pituitaries during the period of most rapid growth, the increase being most dramatic in male rats. Following the period of rapid growth the TSH potency declined in both sexes. On average, the TSH potency in the male's pituitary was about 1.5 times greater than in the female's pituitary.

No single pattern appears to adequately describe the age changes in the potencies of the different hormones present in the anterior pituitary of the species which have been studied. The comparative abundance of data in the rat has lead to a generalization that the potencies of FSH and LH are less in immature animals than in mature animals and less in females than in males. The literature leads one to the conclusion that such a generalization may be only applicable to a few other species.

C. THE DEVELOPMENT OF THE TESTES

The criteria or signs generally taken to denote the onset of puberty are those dependent upon the action of the sex hormones. Hooker (1944) found that in bulls the Leydig cells, which are the major site of testicular androgens, differentiated from intertubular mesenchymal cells and in some cases, had completed this differentiation

by 4 months of age. Thereafter the number of Leydig cells continued to increase until the bulls were 2 years old. From 2 to 5 years of age, the increase in the proportional area occupied by these cells was primarily due to their vacuolation.

Studies on the androgens produced in the testes during pubertal development of the bull reveal that subtle changes in the activities of the Leydig cells also take place. Lindner (1959) and Lindner and Mann (1960) demonstrated that androstenedione (Δ^4-androstene-3,17-dione) is the major steroid present in the testes of calves, and that as sexual maturation proceeds, the amount of testosterone increases so that it becomes the predominant androgen. They observed that the ratio of androstenedione to testosterone changed from 1:1 in calves at 4 months to 1:10 at 9 months. The steroids present in blood taken from the spermatic vein of calves were subsequently examined by Lindner (1961a). He calculated that androstenedione was being secreted by one testis of a 3 month old calf at a rate of 119 ug per hour, and, testosterone at 33 ug per hour. Treatment of the monozygous twin of this animal with human chorionic gonadotropin (HCG) for 50 days prior to the study of the androgen secretion of both animals at 90 days, increased the rate of androstenedione secretion more than eight times but did not alter the testosterone output. Lindner estimated

the androgen output of a normal prepubertal bull calf of 4 to 6 months of age to range from 0.2 to 10.4 mg per day of androstenedione and from 0.6 to 11.1 mg per day of testosterone. It was concluded that the bull testis gains the ability to convert androstendione to testosterone in considerable quantity beginning at the time of puberty. It is of interest to note that in later work, Lindner (1961b) could detect only testosterone in the spermatic vein blood taken from a ram and two boars all between 3 and 4 months of age.

Changes in the intertubular Leydig cells are not the only alterations which occur in the bull testis with advancing age. Abdel-Raouf (1960) divided the reproductive development of bulls into five stages based upon the developmental changes in the seminiferous tubules. The "infantile stage" includes the first 2 months of postnatal life during which period the sex chords are solid and possess only fetal-type cells. During the second stage--the "proliferation stage"--which lasts until 4 to 5 months of age, spermatogonia appear. Lumen formation did not begin until the "prepubertal stage" when primary spermatocytes are present. The "pubertal stage" which lasts from 32 to 44 weeks of age, is characterized by the presence of spermatids and in the later weeks, by sperm. Changes in the "postpubertal" or "adult phase" are primarily quantitative. Some of the variation in the literature quoted by Abdel-Raouf (1960) could possibly be

explained by the results obtained by Knudsen (1954). He found that, in Swedish Red-and-Black bulls, spermiogenesis commenced at 7 months of age, but this process started at different times in different seminiferous tubules in the same bull.

Studies have been made on the changes in the nucleic acid content of rat testes with advancing age. Fujii and Koyama (1962) found that the DNA concentration in rat testes declined rapidly from 2 to 4 weeks of age. This decline continued to 10 weeks of age but at a diminishing rate. Desjardins, Macmillan and Hafs (unpublished data) noted a similar trend and also found that there is a marked increase from birth to 15 days of age. The trends in RNA concentration were similar but not as dramatic as those for DNA. Since the RNA:DNA ratio in the rat testes increased from 3 weeks of age, Fujii and Koyama (1962) suggested that 3 weeks was the age at which the sexual function of the testis commenced.

Attempts have been made to advance the development of the immature testis. Wakeling (1959) injected PMS, HCG, testosterone propionate and human post-menopausal gonadotropin for 7 days into intact male rats which were initially 25 or 28 days of age. All these hormones produced significant increases in the weights of the accessory reproductive organs, indicating that the three gonadotropins used had stimulated testosterone synthesis. However, only

PMS induced precocious differentiation of spermatids and none of the hormones accelerated the process of spermiogenesis. Woods and Simpson (1961) reported that precocious sexual maturity in normal weanling male rats did not result from injections of highly purified ovine FSH and LH, with or without supplementation by other pituitary hormones. However, after hypophysectomy, the two gonadotropins, when given simultaneously, could reduce the age at which spermatids were detected in the seminiferous tubules. They suggested that the immature pituitary was producing an "antigonadotropin."

The age at which the interstitial tissue becomes responsive to exogenous gonadotropins varies between species. Price and Ortiz (1944) demonstrated that the rat testis was responsive to gonadotropin between birth and 6 days of age. However, the maximum response, as measured by the increase in seminal vesicular weight was between 20 and 26 days. By contrast, Mann et al., (1960) found that HCG did not produce significant changes in testicular weight, seminal vesicular weight or seminal vesicular concentrations of fructose and citric acid in calves less than 12 weeks of age. Since exogenous testosterone propionate could produce increases in the last three parameters, they concluded that the inability of HCG to produce any increases was the result of the inability of the bull testis to produce testosterone before 12 weeks of age.

D. THE PITUITARY-TESTIS RELATIONSHIP DURING SEXUAL DEVELOPMENT

While major changes in the pituitary hormones and in the development of testicular function can be demonstrated, the onset of puberty is probably associated with an interaction between these two glands, with the hypothalamus as an intermediary. Harris and Jacobsohn (1952) transplanted pituitaries from immature rats into mature hosts and vice versa. Their results showed that the activity of the pituitary gland depends on the age of the host rather than that of the donor suggesting that the onset of puberty is associated with a change in the regulatory functioning of the hypothalamus.

Such a hypothesis does not preclude the strong possibility that the immature gonad may exert an influence on the immature pituitary gland. In fact, several reports suggest that secretions from the immature rat testis influence the subsequent functional role of the hypothalamus during the first few days of life. Evidence for this assertion is derived from numerous studies involving androgen sterilization of female rats (Gorski, 1966). This sterilization is achieved by injecting a female rat with either testosterone propionate or estradiol benzoate during the first week of life. Following vaginal opening, the rat may have several irregular estrous cycles and then exhibit a constant estrous smear. A recent systematic study by Harris and Levine (1965) led these workers to

conclude that "during the first few days of life in the male rat, the normal mechanism underlying the future patterns of sexual behavior and gonadotropin secretion is organized by the internal secretion of the immature testis into those of the male." Some "organization" of the female hypothalamus may also occur since androgen sterilization is of reduced effectiveness after 6 days of age and is ineffective after 10 days of age. Harris and Levine (1965) suggested that rats of both sexes are born with a sexually undifferentiated central nervous system. The male testis or gonadal hormones can modify this system during the first few days of life. If these modifications do not transpire, the central nervous system is fixed in the form which is capable of functioning in a feminine role.

Even after this "hypothalamic organization" has been completed, the gonads in both sexes of the rat exert a continuous influence on the pituitary, in all probability via the hypothalamus. Numerous studies have shown that gonadectomy in infancy causes an increase in the gonadotropic potency of the pituitary in both sexes. These changes have been confirmed histologically. For example, Libman (1953) and Libman and Jost (1953) castrated male rats at birth and found that pituitary basophils (the source of the gonadotropins) had enlarged by 7 days of age with still further enlargement by 15

days. However, pituitary castration cells similar to those seen in castrated adult males did not appear until 45 days of age.

In the rat, van Rees and Paesi (1955) found that immature males had heavier pituitaries than immature females. The two possibilities considered were that estrogen in the female caused enlargement, or, that androgens in the male kept the gland small. Since gonadectomy caused a 32 percent increase in average pituitary weight in the male but only a 7 percent increase in the female, they concluded that the latter possibility was operative. Recently, Ramirez and McCann (1963) reported that gonadectomy in both immature and mature rats of both sexes caused a marked increase in the plasma levels of LH, but greatest increases were in the females. However, the LH content of the gonadectomized immature females only increased slightly, when compared to the intact immature female, whereas it doubled in the castrated immature male. The net result was that the LH content of the pituitary glands of immature gonadectomized rats of both sexes was similar.

The elevation in plasma LH in ovariectomized female rats was reduced or eliminated when Ramirez and McCann (1963) injected estradiol benzoate. They found that the immature females were two to three times more sensitive to this action of estrogen, than were mature females. These workers hypothesized that the onset of puberty was

associated with a decline in the sensitivity of the hypothalamus to the inhibitory effect of estrogen on the release of pituitary LH. The hypothesis was subsequently extended to male rats as Ramirez and McCann (1965) also demonstrated that the increased plasma levels of LH which follow castration can be more readily reduced by injections of testosterone propionate in immature castrates than in mature castrates. A similar theory was advanced by Donovan and van der Werff ten Bosch (1959) who suggested that in the rat "puberty probably occurs as the hypothalamo-hypophysial system outgrows the depressant action of the minute amounts of gonadal hormone circulating during infancy."

A partial explanation for the results of both groups of workers was possibly provided by Donovan and O'Keefe (1966). They transplanted ovarian tissue to either the kidney or the spleen in both mature and immature female rats. Using this approach it was shown that with increasing age the liver appeared to dramatically increase its capacity to inactivate ovarian hormones. The greatest development in this ability occurred around the time of puberty. Thus, the greater sensitivity of the immature hypothalamus to steroids may be due to the slower rate of inactivation of the steroids by the liver.

However, these data do not preclude the possibility that the sex steroids may also cause a "maturation" of

the hypothalamus. This possibility is based on the results obtained by Ramirez and Sawyer (1965a) who advanced the age of vaginal opening, ovulation and the initiation of estrous cycles in the rat by more than a week by injecting 0.05 ug of estradiol benzoate per 100 g body weight per day. Four injections, starting of the twenty-sixth day of age, were usually sufficient to cause vaginal opening at which point injections ceased. The conclusion reached was that the exogenous estrogen had hastened the maturation of the hypothalamus normally achieved by endogenous estrogen from the ovary.

These changes in the hypothalamus do not appear to be associated with the initiation of the production of luteinizing hormone-releasing factor (LH-RF). Ramirez and McCann (1963) could find no differences in the LH- releasing activity of median eminence extracts from either mature or immature rats. Campbell and Gallardo (1965) and Gallardo and Campbell (1965) reported that median eminence extracts from cattle from 3 months to 2 years of age all contained releasing activity. They reported a slight increase in hypothalamic LH-RF with increasing age, but puberty was not associated with any major change in this activity. It was also reported that day-old rabbits appeared to have the same LH-RF activity as extracts from mature rabbits. However, the assays which were used were not very sensitive. Subsequent work by Ramirez and Sawyer (1966) showed that the normal vaginal opening

and the precocious vaginal opening induced by low dosages of estrogen were associated with a marked rise in hypothalamic LH-RF levels, immediately followed by a dramatic decline similar to that observed after estrus (Ramirez and Sawyer, 1965b) in the estrual cycle fluctuations of LH-RF in the mature rat.

E. THE DEVELOPMENT OF THE ACCESSORY REPRODUCTIVE GLANDS

Although the changes in the pituitary-gonad axis are probably the major factors in initiating the onset of puberty, the more obvious manifestations of puberty involve the dramatic changes exhibited by the accessory reproductive glands. These glands develop under the influence of the increased levels of testosterone produced by the testis. Biochemical measurements on the accessory glands, such as the fructose and the citric acid contents and the nucleic acid changes are very sensitive indicators of the onset of androgen secretion. Rabinovitch and Lutwak-Mann (1951) demonstrated that in castrated rats, the first measurable change induced by an injection of testosterone was a sharp increase in the RNA content of the seminal vesicles. In immature male rats Desjardins, Macmillan and Hafs (unpublished data) found that the RNA and the DNA content of the seminal vesicles increased significantly around 20 days of age, an age considerably less than the accepted age of puberty in the male rat. These changes in nucleic acids apparently precede the

onset of the secretion of fructose, as Porter and Melampy (1952) and Levey and Szego (1955) did not detect significant amounts of fructose until the rats were between 30 and 37 days of age. The latter workers also showed that the production of fructose is demonstrable in the male guinea pig at 20 days of age, long before spermatozoa appear in the testes.

Abdel-Raouf (1960) noted a marked rise in the citric acid and the fructose contents of bull seminal vesicles at 24 weeks of age. The fructose content continued to rise rapidly until 42 weeks of age, whereas the rate of increase in citric acid content was negligible after 36 weeks of age. By comparison, the most rapid increases in testicular weight occurred between 28 and 32 weeks of age. Lindner and Mann (1960) showed that these changes in seminal vesicular activity were related to the increase in androgen production by the testes.

Asdell (1955) stated that in bulls "the seminal vesicles grow more slowly than the body as a whole until the testes have made sufficient growth to supply testosterone which stimulates them." However, Abdel-Raouf (1960) found that the seminal vesicles maintained a relatively constant growth rate to at least 68 months of age.

The development of the different regions of the epididymis of the bull have received little attention in

the literature. Abdel-Raouf (1960) has published the only comprehensive data on the development of this segment of the reproductive tract. He found that the terminal portion of the cauda epididymidis possesses a pseudo-stratified epithelium at birth. The differentiation from a simple columnar to a pseudo-stratified type of epithelium ascended from the cauda to the caput epididymidis and was completed by 32 weeks of age, which was also the age at which sperm appeared in the caput epididymidis. An interesting observation was that the regions of the corpus epididymidis adjacent to the tunica albuginea differentiated first, presumably due to the diffusion of the testicular hormones through the tunic.

Other sections of the male reproductive tract which undergo accelerated development during puberty include the bulbo-urethral glands, the ampullae and the penis. The changes in the penis involve the separation of the glans penis from the penile section of the prepuce, and the development of the sigmoid flexure. Preputial separation is gradual but is complete by 32 weeks of age (Abdel-Raouf, 1960).

MATERIAL AND METHODS

A. EXPERIMENTAL ANIMALS--MANAGEMENT AND SLAUGHTERING PROCEDURES

The basic design for this study involved slaughtering five bulls at birth and five bulls at each month of age thereafter until 12 months of age. Thus, a total of 65 animals were used. Twelve day-old calves were purchased during the months of September and November in 1965 and the months of January, March, May and July in 1966. Five animals from each group were to be slaughtered during August and another five during September (1966). In some groups of 12 bulls, scours caused more than two deaths and replacements had to be purchased. Consequently, fewer animals were killed during August than during September. Five day-old calves were slaughtered on the day of purchase in August and their data was categorized as typical for bull calves at birth.

All calves were sired by registered Holstein bulls from production tested Holstein cows and were purchased from several large dairy farms in lower Michigan. Each animal was tatooed and also identified by a numbered neck-chain. Health records were kept for all animals. Until 4 months of age, each calf was penned separately. Thereafter, the calves were managed communally in a dry-lot

with access to an open shed. Calves from 4 to 8 months of age were housed separately from the older animals.

Late in the afternoon on Sunday or Wednesday, the bulls to be slaughtered the next day were confined to a holding pen without food or water. On the day of slaughter, the animals were loaded at 5:30 A.M. and trucked 2 miles to the Michigan State University Meats Laboratory. Killing had usually commenced by 7 A.M. and was completed by 11 A.M. Each bull was weighed on arrival at the Meats Laboratory.

The routine followed on the killing floor was as follows:

(i) An animal was stunned with a captive-bolt gun and immediately exsanguinated. Four litres of mixed venous and arterial blood was collected where possible in a cold heparinized glass jar and immediately stored at 4° C.

(ii) The pituitary, hypothalamus and a sample of cerebral cortex were dissected free within 10 to 20 min after stunning. The whole pituitary was weighed and then the two lobes were weighed separately. A thin (1.0 mm) mid-saggital section was taken from the anterior pituitary and was preserved in Bouin's fixative, and the remaining portion was weighed and placed in a polyethylene bag on Dry Ice within 20 to 40 min after stunning. The hypothalamus, median eminence and pituitary stalk were finely cubed,

placed in a sample bottle, immersed in a minimum volume of 0.1 N hydrocholoric acid and placed on Dry Ice. The sample of cerebral cortical tissue received similar treatment.

(iii) The thyroid, adrenal glands and thymus were removed between 20 and 45 min after slaughtering, dissected free of fat and connective tissue and weighed. A sample of about 10 g to 20 g from each type of gland was covered with 0.25 M sucrose and placed on Dry Ice for subsequent analysis of DNA and RNA content. A thin section (1-2 mm) from both the thyroid and the adrenal gland was placed in Bouin's fixative for subsequent histological examination.

(iv) The reproductive tract was removed intact. The length of the penis was measured with the sigmoid flexure extended. The epididymis was removed from each testis. The left testis was weighed and immediately stored on Dry Ice for subsequent use in steroidal analyses. The right testis was weighed, the tunica albuginea removed and the testicular parenchyma weighed and sampled for nucleic acid analysis, for histology and for the estimation of gonadal sperm reserves. The latter sample was stored on ice at 0° C.

(v) The left and right epididymides, ductus deferentia, and ampullae were weighed individually. The right half of the tract was ligated between each major segment of the excurrent ducts to minimize the movement of sperm to an adjoining segment, stored at 0° C. and subsequently used for estimating extragonadal sperm reserves. Representative samples were removed from the different sections of the right half of the tract and stored in Bouin's fixative for histological examination.

(vi) The two seminal vesicles were weighed together. One gland was placed on Dry Ice for subsequent estimation of its fructose and citric acid contents. The other gland was used for nucleic acid determinations and for histological measurements. The seminal vesicles, which were the last organs to be dissected, were sampled from 1.0 to 1.5 hours after slaughter.

From one to six bulls were slaughtered on any one day. After slaughtering and dissecting had been completed, all the samples on Dry Ice were transferred to a freezer at -20° C., and the blood and the various samples at 0° C. were placed in a cold room at 4° C.

B. BIOASSAYS OF PITUITARY HORMONES AND LUTEINIZING HORMONE-RELEASING FACTOR:

The anterior pituitaries which had been stored at -20° C., were thawed in a refrigerator at 4° C., weighed to the nearest 0.1 mg and homogenized in a Potter-Elvehjem homogenizer in 10 ml of 0.85% saline. The volume of the homogenate was adjusted to a final concentration of 50 mg pituitary equivalent per ml. The homogenate was centrifuged and the supernatant fluid used in the assays for LH, FSH and GH.

(a) Luteinizing Hormone.--The levels of LH in the pituitaries and blood plasma were measured using the ovarian ascorbic acid depletion method (OAAD) of Parlow (1961). The test rats (Sprague-Dawley strain from Spartan Research Animals, Haslett, Michigan) were injected with 50 IU of PMS (Ayerst Laboratories, "Equinex") at 25 days of age and with 25 IU of HCG (Ayerst "Chorionic Gonadotropin" or Squibb "Follutein") from 58 to 62 hours later. Six days after the HCG injection, each rat was injected intravenously via the femoral vein with 0.5 ml of a test or standard preparation. Four hours later the left ovary was removed and the ascorbic acid concentration was measured in a filtered homogenate containing an equivalent of 10 mg ovarian tissue per ml. At the time of ovariectomy, each rat was subcutaneously injected with 30,000 IU of penicillin G.

Two days later, the rats, which had been communally caged, were re-divided into groups of five. Each rat was again intravenously injected with 0.5 ml of a test preparation and the ascorbic acid concentration was measured in the right ovary. On the first experimental day the rats weighed from 96 to 110 g and the left ovary in one sample of 75 rats weighed 145.1 ± 29.4 g (± SD). The right ovary used on the second experimental day weighed 126 ± 25.4 g.

Each pituitary was assayed at two dose levels with five rats at each level. The two dose levels used were 0.1 mg and 0.4 mg pituitary equivalent per rat. The responses were compared, by the procedure for parallel-line assays (Bliss, 1952), with the responses produced by NIH-LH_B3 at dose levels of 0.4 ug and 1.6 ug per rat. Thirteen pituitaries were assayed each week such that one pituitary from each age group was assayed each week.

It should be emphasized that the left ovary of all rats was used on the first experimental day to assay the lower dose levels of LH or pituitary homogenate, and the right ovary to assay the higher dose levels 2 days later. This is a major modification from the usual procedure in which half the rats are injected with low dose levels and the other half of the rats with high dose levels on both days. That is, when the second ovary is used 2 days after the first ovary, some rats have previously received the lower dose levels and others have received the higher dose levels.

The modification adopted in the present LH assays was the consequence of the results obtained in an experiment which showed that when the first injection contains 1.6 ug or more LH-equivalents, the ovary used on the second day shows a reduced sensitivity to exogenous LH, particularly if the second injection also contains about 1.6 ug LH-equivalents. In this experiment, 75 rats were divided into five equal groups. Each group was subdivided into three smaller sub-groups of five rats. On the first experimental day a group was injected with a dose level of 0, 0.4, 0.8, 1.6 or 2.4 ug NIH-LH-B2 per rat. On the second experimental day one sub-group was injected with 0, 0.4 or 1.6 µg NIH-LH-B2 per rat. The results are tabulated and discussed in the Results and Discussion section of this thesis.

The plasma LH was assayed similarly to the pituitary LH homogenates except that the plasma was extracted to concentrate the LH and then injected at doses of 25 ml plasma-equivalent for first ovaries and 100 ml for second ovaries. The extraction procedure was similar to that outlined by Anderson and McShan (1966). The heparinized whole blood was centrifuged at 13,000 x g for 25 min usually within 7 to 10 hours after the blood had been collected. The plasma was stored at -20° C. until thawing when LH was extracted from a 750 ml sample. In the two youngest age groups, a single animal did not provide 750 ml of

plasma. Consequently, in these two age groups, a pooled plasma sample representing 150 ml from each animal was used.

The details of the plasma-LH extraction procedure were as follows:

(i) The plasma pH was adjusted to 7.4 by the addition of 1N hydrochloric acid; 750 ml cold acetone was slowly added with constant stirring and the mixture was refrigerated overnight.

(ii) The mixture was centrifuged at 14,000 x g for 20 min, the precipitate discarded and the decanted supernatant fluid adjusted to 1500 ml with 50% acetone. Then the pH was reduced to 6.0 with 1N hydrochloric acid and the mixture was refrigerated overnight.

(iii) This mixture was centrifuged at 14,000 x g for 20 min. One liter of cold acetone was slowly added to the decanted supernatant fluid (resulting in a 70% acetone mixture) and this mixture was refrigerated overnight.

(iv) This 70% acetone mixture was centrifuged at 14,000 x g for 20 min and the supernatant fluid was decanted and discarded. The precipitate (containing LH) was dissolved in water and the centrifuge bottles were each washed three times. The washings were pooled and

stored for 2 to 4 days at -20° C. before lyophilization.

(v) Immediately before bioassay, each lyophilized sample was dissolved in 3.75 ml 0.85% saline and 0.75 ml of this solution was further diluted with 2.25 ml saline. These two saline solutions contained 25 ml or 100 ml plasma equivalents, respectively, per 0.5 ml.

All centrifugations during this extraction procedure were performed at 4° C. The efficiency of the extraction procedure was determined by adding known amounts of NIH-LH-B3 to plasma samples and determining extraction losses. The possibility that the extraction of vasopressin with LH may have influenced the ovarian ascorbic acid response, as suggested by Taleisnik and McCann (1960), was tested by the addition of known amounts of "Pitressin" (Parke-Davis) to plasma samples which were extracted in the above manner and the results compared to a plasma sample which contained no "Pitressin."

(b) Luteinizing Hormone-Releasing Factor (LH-RF).-- The preparation of the hypothalami prior to the bio-assay of LH-RF was based upon the method of Nikitovitch-Winer et al., (1965). These workers showed that LH contamination of hypothalamic extracts could be eliminated by boiling and dialysis. The following procedure was adopted to assay LH-RF:

(i) The chopped hypothalami which had been stored immersed in 0.1 N hydrochloric acid at -20° C. were thawed at 4° C. and the five samples from each age group were pooled. The pooled tissues were homogenized in their storage media (total volume of approx. 50 ml). Each homogenate (and acid washings) were adjusted to 150 ml and centrifuged at 10,000 x g for 10 min. The precipitate was discarded.

(ii) The supernatant fluid was incubated in a water bath at 100° C. for 10 min, cooled and dialyzed against 0.1 N hydrochloric acid for 12 hours.

(iii) The dialysate was neutralized to pH 6.0 with 2N sodium hydroxide, frozen and lyophilized.

(iv) Immediately before assay, the lyophilized extract was dissolved in 5.0 ml of water, and 2.0 ml of this solution was re-diluted with 2.0 of water.

Rats prepared similarly to those for the routine LH-assay, were injected intravenously with a high dose level of 0.25 hypothalamic equivalents. Four hours after a rat was injected, the ovarian ascorbic acid concentration was measured and the ovarian ascorbic acid response to LH-RF was compared to the values obtained from rats injected with 0.5 ml 0.85% saline. Other "controls" included similarly treated cerebral cortical extracts, NIH-LH-B2 which was boiled in 0.1 N acid, dialyzed and

injected at a dose level equivalent to 20 ug LH per rat (if destruction and removal had been completely ineffective), and rats injected with untreated NIH-LH-B2 at a dose level of 0.4 ug LH per rat. NOTE: Since the rats injected with high dose levels of hypothalamic extract experienced shock due to the saline hypertonicity of the extract, an additional preparative step is recommended. After dialysis and neutralization to pH 6.0, the salt should be removed by the use of a "bio-gel" column.

(c) Follicle Stimulating Hormone (FSH).--The bovine pituitary contains little FSH relative to LH and relative to pituitary FSH potencies in other species. Consequently, a low dose level equivalent to at least 50 mg of bovine pituitary should be injected into each rat for the ovarian weight augmentation assay (Steelman and Pohley, 1953). In this laboratory, indices of precision in FSH assays tend to be large and variable and the slopes of the dose-response curves are relatively small (Desjardins et al., 1966). To improve the precision of this assay, ten rats were used at each dose level of each test solution instead of the usual number of five per dose level, and the rats received 50 IU HCG concomitantly with each dose level of each test solution instead of 20 IU. The former modification precluded assaying each individual pituitary due to insufficient material. Since Desjardins (1966) found that there

was little variation in the FSH potency among heifers within an age group, or even among age groups, a more precise estimate of the average potency within an age group was considered to be more important than measuring each individual pituitary with reduced accuracy.

Therefore, the following assay procedure was adopted:

(i) Equal aliquots of the pituitary homogenates (described previously) from the five bulls within an age group were pooled. Ten rats were each injected subcutaneously with 50 mg pituitary equivalent and ten with 100 mg pituitary equivalent from each age group. At each dose level, 50 IU HCG (Squibb "Follutein") was added to the pituitary extract and the total dose was administered in nine separate injections of 0.40 ml, each rat receiving three injections per day for 3 consecutive days. Injections commenced when the female Sprague-Dawley rats were 22 days of age.

(ii) On the morning following the last 8:00 P.M. injection (twenty-fifth day of age), the ovaries were removed and weighed. The weight responses of the ovaries to the pituitary homogenates were compared to responses in rats receiving standard preparations of 50 ug or

100 ug of NIH-FSH-S3. Potency estimates were calculated by the slope ratio procedure outlined by Bliss (1952).

The pituitaries from bulls less than 3 months of age were too small to provide sufficient material for the injection of ten rats at each dose level. In these cases the number of rats was reduced to five per dose level.

(d) Growth Hormone (GH).--The assay used for estimating GH potency was the tibia response in hypophysectomized female rats (Evans et al., 1943). As for the FSH assays, pooled samples of the pituitary homogenates from the five bulls within each age group were used. The rats (supplied by Hormone Assay Laboratories, Chicago) were hypophysectomized at 28 days of age and were delivered to our laboratory at 39 days of age. The test solutions were injected subcutaneously twice daily for 4 days. The injections commenced on the twelfth day after hypophysectomy. During the 4-day injection period, each rat received a total of either 4 mg or 16 mg pituitary equivalent and four rats were used with each dose level from each pooled homogenate.

The standard preparations used were 25 ug NIH-GH-B9 plus 25 ug NIH-TSH-B3 and 100 ug of both hormones per rat. The thyroid stimulating hormone (TSH) was added to the GH standard preparations because TSH and GH have a

synergistic action on the tibia response of a hypophysectomized female rat. However, the TSH augmentation eventually plateaus, (Schooley et al., 1966) and the ratio of 1 ug TSH per 1 ug GH should compensate for the presence of TSH in the pituitary homogenates.

Twenty-four hours after the last injection of pituitary homogenate, a tibia was taken from each rat, dissected free, split at the proximal end in a sagittal plane, and fixed in neutralized 10% formalin. The tibias were stained according to the procedure outlined by Evans et al., (1943) and the width of the uncalcified cartilage measured at eight points using a micrometer eye-piece. The average width was used in calculating potency estimates by the method for parallel-line assays (Bliss, 1952).

C. GONADAL AND EXTRA GONADAL SPERM RESERVES

The technique used for the direct estimation of the gonadal and extra-gonadal sperm content of bulls which were 5 months of age or older was an adaption of the procedure developed by Amann and Almquist (1961). The tissues used for this purpose were homogenized between 2 and 6 hours after a bull had been slaughtered. The homogenates were stored overnight at 4° C. and the sperm concentration of each homogenate was estimated hemocytometrically the next day.

Before homogenization of a testicular sample, that tissue which had been in direct contact with the

polyethylene bag used for storage was removed and from 15 to 25 g of parenchymal tissue was weighed, diced and homogenized for 2 min in a Waring Blendor with 200 ml 0.85% saline. If the sample weighed less than 15 g, it was homogenized with only 100 ml 0.85% saline.

The caput, corpus, and cauda epididymides were isolated, weighed, diced and individually homogenized in saline for 2 min. The ductus deferentia were similarly treated, except that they were homogenized for 3 min. The volumes of 0.85% saline used for homogenization were 50 ml for the caput and cauda epididymides and the ampullae, and 25 ml for the corpus epididymides and ductus deferentia. As indicated above, these segments were from the right half of each reproductive tract.

The sperm concentrations in the homogenates were estimated, in duplicate, hemocytometrically using phase contrast microscopy. Consistent with accurate hemocytometric enumeration, it was necessary to dilute some homogenates to a concentration of sperm such that from 30 to 150 sperm were counted within the grid of the hemocytometer.

D. BIOCHEMICAL PARAMETERS IN THE TESTIS AND SEMINAL VESICLES

The biochemical parameters measured in both organs were the concentrations of the nucleic acids (DNA and RNA). The levels of fructose and citric acid were

measured only in the seminal vesicles. The samples, which had been stored in 0.25 M sucrose at -20° C., were thawed at 4° C. and homogenized to produce final concentrations of 50 mg of tissue per ml. A 2 ml sample of each tissue homogenate was used in the analytical procedure outlined by Tucker (1964) to determine DNA and RNA concentrations, total DNA and total RNA per organ and the RNA/DNA ratios. The RNA parameters were used as indicators of protein synthetic activity and DNA as a measure of cell numbers.

The seminal vesicular tissue used for fructose and citric acid determinations was diced before it had thawed so that the loss of seminal vesicular fluid was minimized. The measurements of both constituents were based on modifications of the procedures described by Lindner and Mann (1960).

The analytical procedure for fructose was as follows:

(i) Two grams of tissue was homogenized for 2.5 min in 38 ml 80% ethanol and centrifuged at 10,000 x g for 10 min. The precipitate was discarded and 10 ml of the supernatant fluid was evaporated almost to dryness at 20° C.

(ii) Water was added to the residue to give a total volume of 8.0 ml. One ml 5% $ZNSO_4.7H_20$ and 1 ml 0.3 N $Ba(OH)_2$ were added and the mixture centrifuged at 10,000 x g for 10 min.

(iii) Each of three aliquots (from 1.00 ml to 0.05 ml) of the supernatant fluid was adjusted to 2.0 ml with water. Smaller aliquots were used with the older bulls than with the young bulls. To each aliquot was added 2.00 ml 0.1% resorcinol in absolute ethanol and 6.00 ml 30% hydrochloric acid. After mixing, the samples were incubated for 10 min at 80° C. and then cooled. Optical densities were read at 490 mu and fructose content of the aliquot calculated from a standard curve derived from pure fructose. The aliquot with a transmittency closest to 50% was used in the subsequent calculations of fructose concentration.

The analytical procedure for citric acid was:

(i) Two grams of tissue was homogenized for 2.5 min in 38 ml 10% trichloroacetic acid (TCA) and the homogenate was centrifuged at 10,000 x g for 10 min.

(ii) Three aliquots of the supernatant fluid (from 1.00 ml to 0.20 ml) were adjusted to 1.0 ml with 10% TCA. Eight ml of acetic anhydride was added to each adjusted aliquot and after mixing, the mixture was incubated at 60° C. for 10 min. After cooling, 1.00 ml pyridine was added to each tube. The pyridine mixtures were incubated at 60° C. for 40 min. The

transmittency of the cooled mixture was read at 400 mu and the results compared to a standard curve obtained using pure citric acid. The aliquot with the transmittency closest to 50 percent was used in subsequent calculations.

In the cases where the total amount of tissue was less than 4.0 g, the homogenization volumes were reduced so that the evaporated extract for fructose contained the equivalent of 0.5 g of tissue and the TCA supernatant fluid for citric acid analysis contained 50 mg of tissue equivalent per ml of supernatant.

E. HISTOLOGICAL DETERMINATIONS

Sections from the thyroid, adrenal, testis and each of the three segments of the epididymis, the mid-section of the vas deferens, the mid-section of the ampulla, and seminal vesicles were stained with Harris's hemotoxylin and eosin. The width of the adrenal zona glomerulosa and the combined width of the zonas reticularis and fasiculata were measured in each of four fields from the adrenal gland of each bull. Four measurements were made of the heights of the luminal epithelial cells in the thyroid, seminal vesicles, ampulla, ductus deferens and the caput, corpus and cauda epididymides. The tubular diameters of the ductus deferentia and of each of the three segments of the epididymides were also measured in quadruplicate.

The diameter of four seminiferous tubules was measured in each testicular section and these sections were also studied for the presence or absence of lumina in the tubules and for the presence of terminal stage spermatids. The latter observations were made at a magnification of 540 x under oil.

Midsagittal sections of the anterior pituitary gland from 10 to 12 u thick were stained by the periodic acid Schiff technique as outlined by Jubb and McEntee (1955). The sections were projected so that the proportional areas of the basophilic and acidophilic regions could be measured.

RESULTS AND DISCUSSION

A. BODY WEIGHT CHANGES

One method for comparing the similarities and differences between experimental populations is to examine live weights and body weight gains. The presumed basis for such a comparison is that extreme environmental conditions will be clearly reflected by these parameters. However, a proportional extrapolation of any differences in body weights to the parameters for reproductive development may not be strictly justifiable.

The monthly averages for the live weights of the bulls used in the current study are plotted, with standard errors in Fig. 1. The calculated regression equation for these data is $\hat{Y} = 22.5 + 26.5X$ ($\hat{Y}$ = body weight in kg; X = age in months). The lack of fit component of the error term of the analysis of variance was not significant ($p = 0.20$). However, the average weight of the five bulls killed at 1 to 2 days of age was significantly greater than the body weight calculated by use of the above equation (35.3 vs. 22.5 kg) ($p < 0.05$).

The bulls used in this study weighed less at birth and 1 month of age, than the Holstein bulls of comparable age measured by Morrison (1956) and Bratton et al., (1959). Apart from these two ages, the values obtained in this

TABLE 1.--Comparative body weights of bulls to one year of age.

Age	Michigan[a] State Values	Morrison's[b] Values	Cornell[c] Values	Abdel-[d] Raouf's Values
(months)	----------	kg	----------	
Birth	35.3	43.6	42	38
1	43.7	54.4	57	75
2	72.5	71.3	72	112
3	96.7	--	88	132
4	138.2	120.3	106	152
5	149.6	--	132	174
6	178.8	181.8	159	190
7	198.5	--	183	205
8	229.5	240.1	210	220
9	263.7	--	234	235
10	285.4	294.2	260	246
11	322.7	--	287	258
12	341.1	350.5	310	265

[a]Results from current study.

[b]Derived from data of Morrison (1956).

[c]Derived from data of Bratton et al., (1959).

[d]Derived from data of Abdel-Raouf (1960).

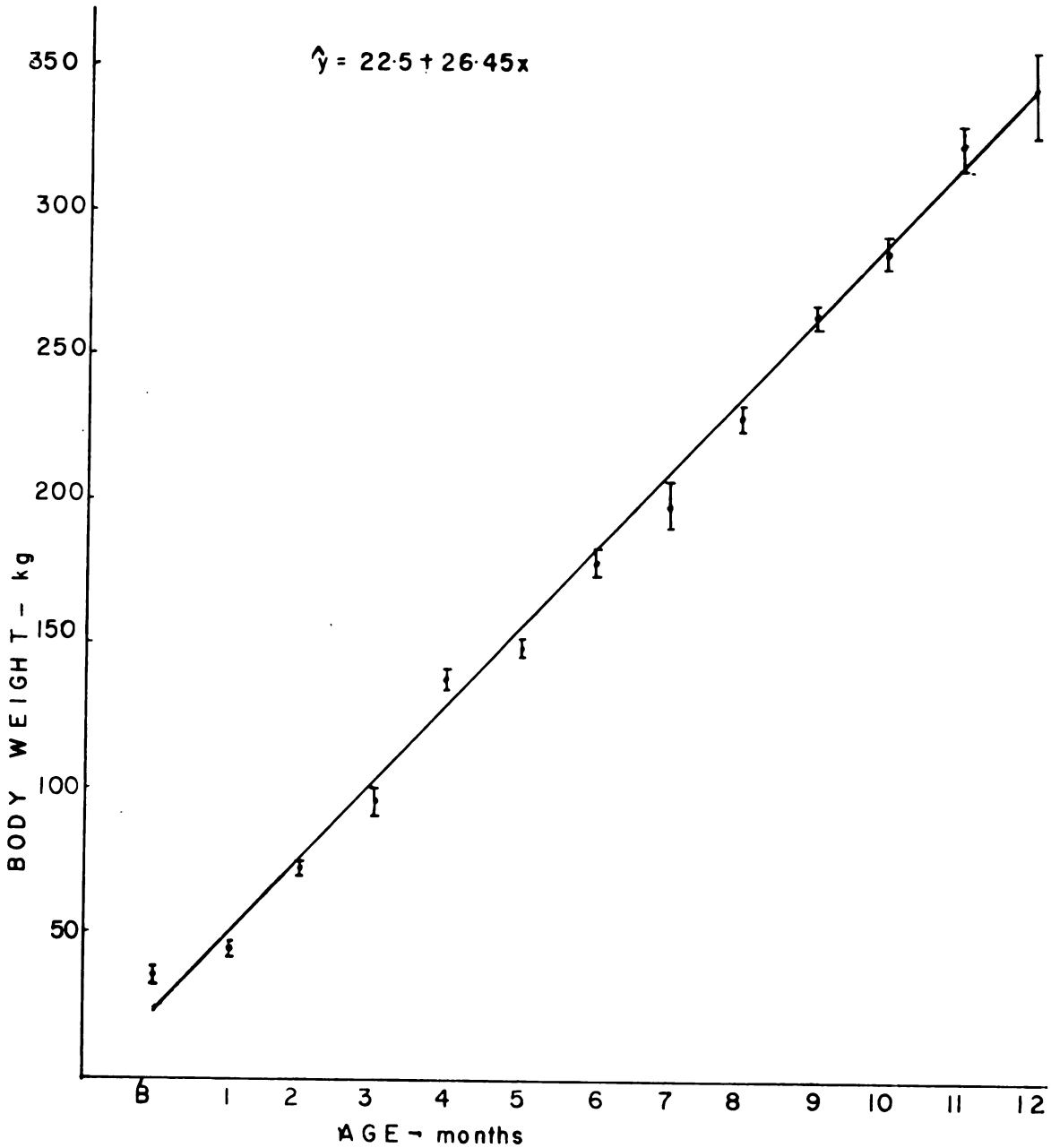

Figure 1.--Average body weight (ISE) of Holstein bulls to 12 months of age and the regression equation describing the increase in body weight.

study are similar to those quoted by Morrison (1956), and are 15 to 30 kg greater than those reported by Bratton et al., (1959). In contrast to Holstein bulls, the Swedish Red-and-White bulls used by Abdel-Raouf (1960) followed a quadratic growth curve. Consequently, this Swedish breed is heavier from 1 to 7 months of age but thereafter the rate of body-weight gain declines and at 12 months of age Holstein bulls are over 80 kg heavier. This difference in the growth curves of the two breeds suggests that bulls of the Swedish breed attain maturity, in terms of body weight, at a younger age than Holstein bulls.

Since a linear equation adequately describes the increase in the body weight of the Holstein bulls used in this study, it can be concluded that the period of puberty is not associated with any noticeable changes in the rate of increase in body weight.

B. CHANGES IN THE PITUITARY AND THE PITUITARY HORMONES

(a) Pituitary Weight.--The data for pituitary weights are summarized in Table 2. The standard errors of the monthly means are recorded in Appendix Table I with the data for individual bulls. The weight of the posterior pituitary increases three-fold from birth to 12 months of age, but the rate of increase is relatively constant. Consequently, the month-to-month variations in the weight of the whole

TABLE 2.--Average weights of the whole pituitary and the posterior and anterior pituitary lobes in bulls and the anterior pituitary lobe in heifers.

Age	Bulls			Heifers[a]
	Whole Pituitary	Posterior Pituitary	Anterior Pituitary	Anterior Pituitary
(months)	g			
Birth	0.57	0.13	0.39	0.44
1	0.58	0.14	0.39	0.48
2	0.78	0.19	0.56	0.66
3	0.99	0.22	0.73	0.85
4	1.15	0.28	0.80	0.90
5	1.21	0.25	0.93	1.02
6	1.28	0.26	0.96	1.22
7	1.18	0.32	0.80	1.26
8	1.22	0.30	0.87	1.37
9	1.68	0.35	1.24	1.48
10	1.63	0.35	1.19	1.40
11	1.83	0.39	1.33	1.40
12	1.77	0.41	1.27	1.40

[a]Data from Desjardins (1966).

pituitary are largely due to the weight changes of the anterior pituitary. The monthly averages for the weight of the anterior pituitary are graphically represented in Fig. 2. The growth of this gland is linear from 1 to

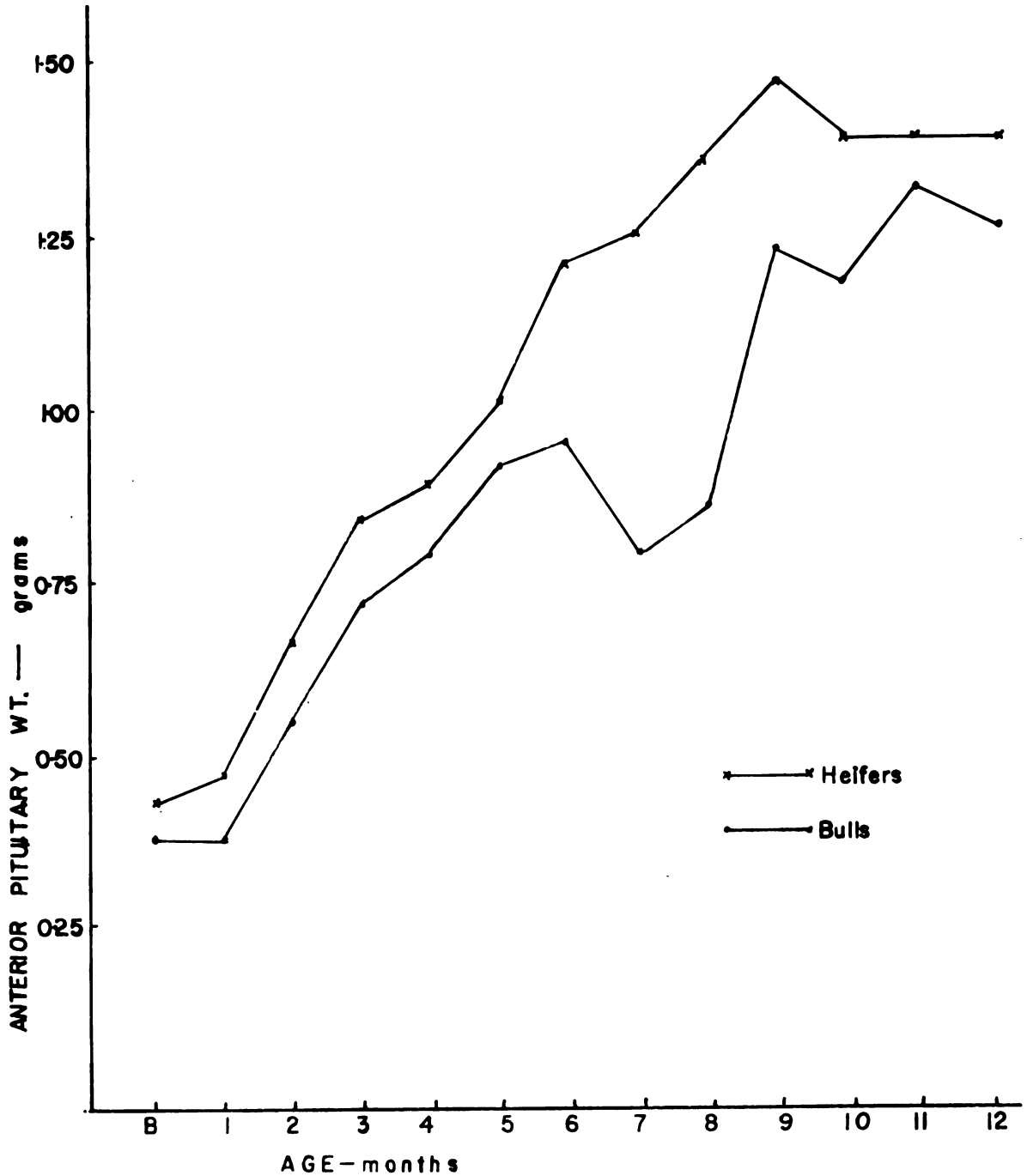

Figure 2.--Changes in the weight of the anterior pituitary in Holstein bulls and heifers.

6 months, but decreases at this age from 0.96 g to 0.80 g at 7 months, and then increases dramatically to 1.24 g at 9 months. Changes from 9 to 12 months are small. An analysis of variance showed that a significant linear component ($p < 0.001$) accounted for 90 percent of the variation among months of age. However, the inflections at 7 and 8 months were associated with a significant quintic component ($p < 0.01$) which accounted for an additional 4 percent of the variation.

This decline in the weight of the anterior pituitary during puberty has not been reported in previous studies in other animals. However, Ryan and Philpott (1967) showed that daily injections of testosterone or androstenedione could reduce the average pituitary weight of mature castrated rats to less than that in intact rats of comparable age. A possible explanation for the decline in the pituitary weight during puberty in Holstein bulls is that the anterior pituitary becomes more sensitive to increasing titers of testosterone at 7 to 8 months of age.

Abdel-Raouf (1960) considered that the increase in the hypophysial weight of Swedish Red-and-White bulls followed a quadratic growth curve. However, there was considerable variation around his line of best fit. From 1 to 6 months of age average pituitary weights were heavier in the Swedish breed than in the Holstein bulls. From 7 to 12 months of age the difference was reversed and at

12 months of age the average hypophyseal weights were 1.77 g and 1.50 g for the Holstein and Swedish bulls, respectively. Although Abdel-Raouf does not comment on the fact, the average pituitary weights of his bulls declined from 5 months to 9 months of age. Reece and Turner (1937) grouped their young animals into three categories --calves, including animals of both sexes to 3 months of age; heifers, steers or bulls from 4 to 10 months of age; and open and pregnant heifers, steers and bulls from 11 to 23 months of age. The anterior pituitaries of the 28 bulls in the 4 to 10 month category averaged 0.64 g with a range from 0.34 g to 1.00 g. All the monthly averages of the Holstein bulls used in the present study and included in this age range exceeded the average quoted by Reece and Turner (1937).

Although Reece and Turner (1937) concluded that there was no difference between the bulls and heifers of comparable age in anterior pituitary weight, a comparison (Table 2 and Fig. 2) of the data obtained in the present study with that recently reported for heifers by Desjardins (1966) reveals that the anterior pituitary weights in the bulls are consistently less than those of heifers. A similar sexual difference has been reported in rats (van Rees and Paesi, 1955) and it was shown to be due to the restrictive effects of androgens on pituitary growth. A similar explanation may be applicable in dairy cattle as

the data presented by Reece and Turner (1937) show that the anterior pituitary is heavier in steers than in bulls of comparable age.

(b) Luteinizing Hormone (LH).--Levels of LH were measured in the anterior pituitary and in blood plasma, the latter being taken as a measure of LH-release from the pituitary. The LH potency was estimated using an assay which incorporated a major modification from the normally accepted routine. This modification was to inject each rat with a low dose of the standard or test extract on the first experimental day (DAY 1) and with a high dose on the second experimental day which was 2 days later (DAY 2). Details were presented in the section for Materials and Methods.

Before the adoption of this modification, an experiment was designed to determine:

(i) whether an injection of LH on DAY 1 altered the concentration of ascorbic acid in the ovary or the responsiveness of the ovary to LH on DAY 2;

(ii) whether an injection of LH on DAY 1 influenced the among rat variation on DAY 2;

(iii) whether LH given on DAY 1 altered the weight of the ovary on DAY 2; and

(iv) whether the response to LH is influenced by ovarian weight.

The data obtained on DAY 1 of the experiment are presented in Table 3. The "time of injection" classification was included as a factor contributing to the total variation because the five doses were each administered to groups of five rats in an ascending sequence which was repeated three times. Thus, there was a total of 15 rats (three groups of five rats) at each dose level and the only difference between the three groups within a dose level was that the second and third groups were injected 50 and 100 min, respectively, after the first group. The comparison of groups within an LH dose level measures trends which may be associated with diurnal variation.

TABLE 3.--Ascorbic acid concentrations[a] after injections of LH on DAY 1.

Time of Injection[b]	ug LH per rat[c]					Mean
	0	0.4	0.8	1.6	2.4	
1	9.71	6.90	5.39	4.52	3.69	6.04
2	8.20	6.42	5.37	3.87	3.44	5.46
3	8.26	6.71	4.91	3.73	2.80	5.28
Mean	8.72	6.68	5.22	4.04	3.31	

[a]ug ascorbic acid per ml ovarian homogenate (equivalent to 10 mg ovarian tissue).

[b]Interval between the injection of groups within each dose level was 50 min.

[c]NIH-LH-B2 injected in 0.5 ml saline.

The mean responses to LH on DAY 1 and their standard errors (for n = 5) are presented graphically as line A in Fig. 3. The analysis of variance showed that differences among LH doses and among times of injection are significant ($p < 0.001$ and $p < 0.005$, respectively) but their interaction also approaches significance ($p = 0.06$). The effect of the time of injection follows a linear decline ($p < 0.001$) which averages 0.38 ug ascorbic acid per ml ovarian homogenate per hour. This decline was not anticipated but a search of the literature revealed that it has previously been reported in uninjected pseudopregnant rats by Stevens et al., (1964) and by de la Lastra and Croxatto (1965). The former group found that the ascorbic acid concentration was greatest at 1 P.M. and declined to 8 P.M. Ovariectomy was always performed on the rats used for the LH assay in our laboratory between 12:30 P.M. and 4:30 P.M.

Because of the interaction between the dose of LH and the time of LH injection in the present experiment, a correction factor applicable to all doses could not be calculated. If the diurnal decline in the concentration of ovarian ascorbic acid is constant within each LH dose level, the effect on LH potency estimates would be minimized with lower doses of LH because of their log-dose relationship.

In the data obtained for the second ovary on DAY 2 (Table 4), the effects of LH dose level are confounded by

the effects of time of injection. Nevertheless, the most dramatic result is the effect of a dose of LH on DAY 1 on the ascorbic acid concentration of the saline injected rats on DAY 2. The rats injected with 0 ug LH on both days had an average ascorbic acid concentration of 9.65 ug per ml on DAY 2. If the rats were injected with from 0.4 to 2.4 ug LH on DAY 1 but only 0 ug LH on DAY 2, the ascorbic acid concentration on DAY 2 was less than 9.65 ug per ml ($p < 0.001$). Similarly, the average ascorbic acid concentration for the rats injected with 0.4 ug LH on Day 2 was greater if the rats had been injected with 0 ug LH on DAY 1 ($p < 0.01$).

TABLE 4.--Ascorbic acid concentrations[a] after injections of LH on DAY 2 following varied LH treatments on DAY 1.

ug LH[b] per rat on DAY 2	ug LH[b] per rat on DAY 1				
	0	0.4	0.8	1.6	2.4
0	9.65	6.76	6.02	7.89	5.33
0.4	6.44	5.80	5.43	5.43	5.60
1.6	3.70	3.41	3.70	3.95	2.46

[a]ug ascorbic acid per ml ovarian homogenate (equivalent to 10 mg ovarian tissue).

[b]NIH-LH-B2 injected in 0.5 ml saline.

Components of variance analyses (Table 5) revealed a lower value for dose variance ($\hat{\sigma}^2_D$) on DAY 2 than on DAY 1. This is largely due to the fact that the three LH dose levels on DAY 2 ranged from 0 ug to 1.6 ug per rat but the five LH dose levels on DAY 1 ranged from 0 ug to 2.4 ug per rat. The variance among groups within doses ($\hat{\sigma}^2_{G:D}$) showed a six-fold increase from DAY 1 to DAY 2. This increase is no doubt due to the fact that on DAY 2 each group within a DAY 2 dose level had received a different dose level on DAY 1. If this variance component ($\hat{\sigma}^2_{G:D}$) is partitioned from the error term ($\hat{\sigma}^2_W$) in the analysis of DAY 2 data, the latter component is identical on both days. If, however, the dose level on DAY 1 is not considered when analyzing DAY 2 data, the error component ($\hat{\sigma}^2_W + \hat{\sigma}^2_{G:D}$) will be greater on DAY 2. The net effect of this increased error variance is to decrease the precision of the assay.

TABLE 5.--Variance estimates for the ovarian ascorbic acid concentrations on DAY 1 and DAY 2.

Source	Symbol	DAY 1 Estimate	DAY 2 Estimate
Dose	$\hat{\sigma}^2_D$	4.59	3.23
Groups within a dose	$\hat{\sigma}^2_{G:D}$	0.18	1.06
Rats within a group	$\hat{\sigma}^2_W$	0.32	0.32

Since researchers in our laboratory routinely use LH dose levels of 0.4 and 1.6 ug per rat, these two dose levels in the present experiment warrant closer consideration (see Table 6 and Fig. 3). Therefore, the data in Tables 3 and 4 were rearranged to predict the validity of future assays of different designs. The usual procedure is to inject high and low doses on each day. With this procedure, the DAY 1 data and the DAY 2 data are represented by lines A and D respectively (Fig. 3). The decline in slope from 4.38 on DAY 1 (line A) to 3.21 on DAY 2 (line D) is primarily due to the reduced responsiveness of rats which received 1.6 ug LH on DAY 1. That is, on DAY 2, the rats which had received 0.4 ug LH on DAY 1 had a slope of 3.96 but the rats which had received 1.6 ug LH on DAY 1 had a regression of only 2.46 (see lines B and C of Fig. 3). This suggests that the rats which had received the higher dose level of LH on DAY 1 were less sensitive in their response to LH on DAY 2 than the rats which had received the lower dose level of LH on DAY 1.

Rats, which were injected with 0.4 ug LH on DAY 1 and 1.6 ug LH on DAY 2 (line E) produced results with a regression of 5.49 which is considerably greater than the other assay designs outlined in Table 6. These data were the basis for the modification of the conventional LH assay as used in the study. This modification is considered justified when the majority of the test solutions

have low dose levels with no more than 0.8 ug LH equivalent per rat.

Correlation coefficients between ovarian ascorbic acid concentration and ovarian weight on DAY 1 were 0.44, 0.15, 0.21, -0.06 and 0.04 with dose levels of 0, 0.4, 0.8, 1.6 and 2.4 ug LH per rat, respectively. Only the first correlation coefficient was statistically significant ($p < 0.05$), confirming that the concentration of ovarian ascorbic acid following an injection of LH is not modified by ovarian weight. The average ovarian weight declined from 145.1 $\pm$ 294. ($\pm$ SD) on DAY 1 to 128.6 $\pm$ 25.4 on DAY 2, but injections of LH did not reduce or modify the decline.

TABLE 6.--Slopes and indices of precision (λ) with different assay designs for estimating potencies of LH.

Design	Lack of Parallelism	Slope	λ	Line on Figure 5
0.4 ug and 1.6 ug on DAY 1	$p > 0.25$	4.38	0.08	A
Same as DAY 1 on DAY 2 following 0.4 ug on DAY 1	--	3.96	0.13	B
Same as DAY 1 on DAY 2 following 1.6 ug on DAY 1	--	2.46	0.07	C
Same as DAY 1 on DAY 2 ignoring DAY 1 treatment	$p = 0.10$	3.21	0.11	D
0.4 ug on DAY 1 and 1.6 ug on DAY 2	--	5.49	0.17	E

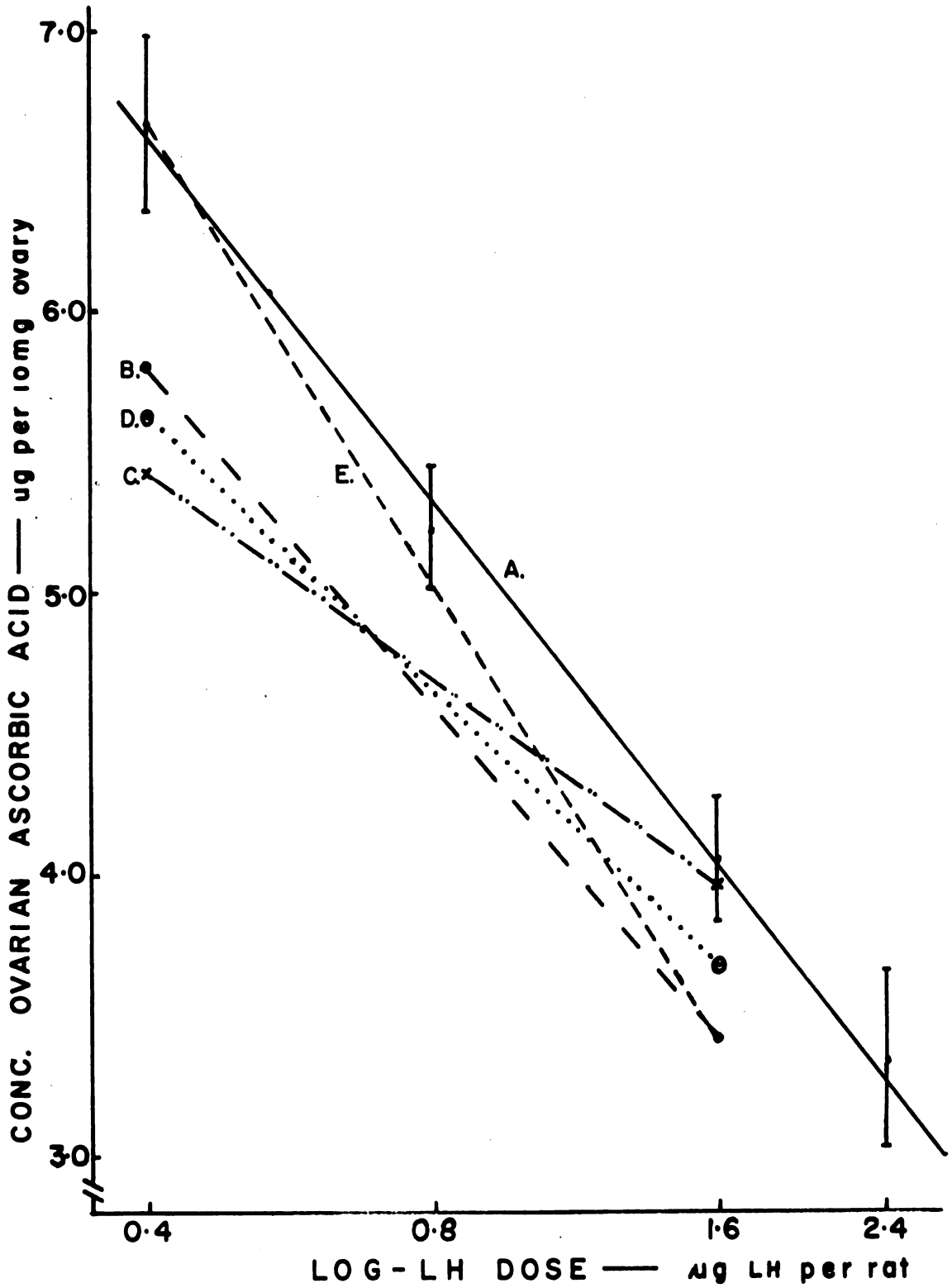

Figure 3.--Dose-response relationship between LH and ovarian ascorbic acid concentration with different injection regimes (see Table 6).

In summary, this preliminary experiment showed that:

(i) An injection of LH on DAY 1 reduces the concentration of ovarian ascorbic acid on DAY 2, but does not alter the responsiveness of the ovary to LH on DAY 2 unless the dose level on DAY 1 exceeds 1.6 ug LH equivalent per rat;

(ii) Injection of a wide range of dose levels of LH on DAY 1 increases the apparent among rat: within dose variation on DAY 2, unless the DAY 1 treatment effect is removed from the error variance when among rat:within dose variation is similar on both days;

(iii) Injections of LH on DAY 1 do not modify the loss of weight by the remaining ovary used on DAY 2;

(iv) The response of the ovary to LH, as measured by ascorbic acid concentration is not influenced by ovarian weight; and

(v) The variances, slopes and indices of precision indicate that the modified assay is superior to the original design.

These were the conclusions which justified the modification of the assay. The indices of precision obtained in the 5 weeks during which the pituitaries from the 65 bulls were assayed were 0.10, 0.17, 0.13, 0.13 and 0.11, respectively. These low values show that the use of the modified assay was justified.

The estimate of the LH concentration (LH potency) in each pituitary derived from the ovarian ascorbic acid changes produced by two dose levels of pituitary extract was used to calculate the LH content of the anterior pituitary. Referral to Fig. 4 shows that the LH potency has a striking peak at 1 month of age, whereas the total pituitary content of LH increases irregularly to reach a maximum value at 6 months of age. Consequently both parameters should be considered when the objective is to relate hormonal changes to reproductive development. The differences between monthly averages for both pituitary LH parameters (Table 7) are significant ($p < 0.001$). Analysis of variance of the potency data showed that all the polynomial components from linear to quintic were significant but none accounted for more than 13 percent of the total among months variation. The data for total LH content had a significant linear trend ($p < 0.01$) which accounted for 43 percent of the variation of this parameter among months.

There were no monthly fluctuations in either parameter which could be interpreted as being indicative of the onset or termination of puberty.

Desjardins (1966) showed that the average LH potency of heifers' pituitaries also fluctuated from month to month but the heifers showed considerably greater variation within almost every age group (Table 7). By contrast, the lowest average potency in heifers was at 1

TABLE 7.--Changes in the pituitary potency and pituitary content of luteinizing hormone in bulls and the comparable potency data in heifers.

Age	Bulls		Heifers[a]
	Potency	Content	Potency
(months)	(ug LH/mg)[b]	(mg LH/pituitary)	(ug LH/mg)[c]
Birth	0.76 ± 0.08[d]	0.30 ± 0.06	2.44 ± 0.74
1	4.88 ± 0.46	1.91 ± 0.22	2.07 ± 0.25
2	2.34 ± 0.38	1.36 ± 0.30	5.79 ± 0.70
3	3.16 ± 0.39	2.31 ± 0.28	9.09 ± 3.05
4	2.44 ± 0.15	1.93 ± 0.18	4.48 ± 1.75
5	2.97 ± 0.43	2.78 ± 0.47	8.63 ± 1.41
6	2.89 ± 0.49	2.83 ± 0.57	4.46 ± 1.01
7	2.47 ± 0.48	1.95 ± 0.38	10.19 ± 3.53
8	1.87 ± 0.27	1.64 ± 0.25	7.25 ± 1.78
9	2.05 ± 0.21	2.47 ± 0.17	5.61 ± 2.49
10	2.28 ± 0.31	2.76 ± 0.48	2.88 ± 1.85
11	2.12 ± 0.35	2.79 ± 0.53	5.08 ± 1.30
12	1.96 ± 0.07	2.51 ± 0.15	6.79 ± 1.55

[a]Data from Desjardins (1966).

[b]ug NIH-LH-B3 equivalent per mg fresh pituitary

[c]ug NIH-LH-B2 equivalent per mg fresh pituitary.

[d]Mean ± SE.

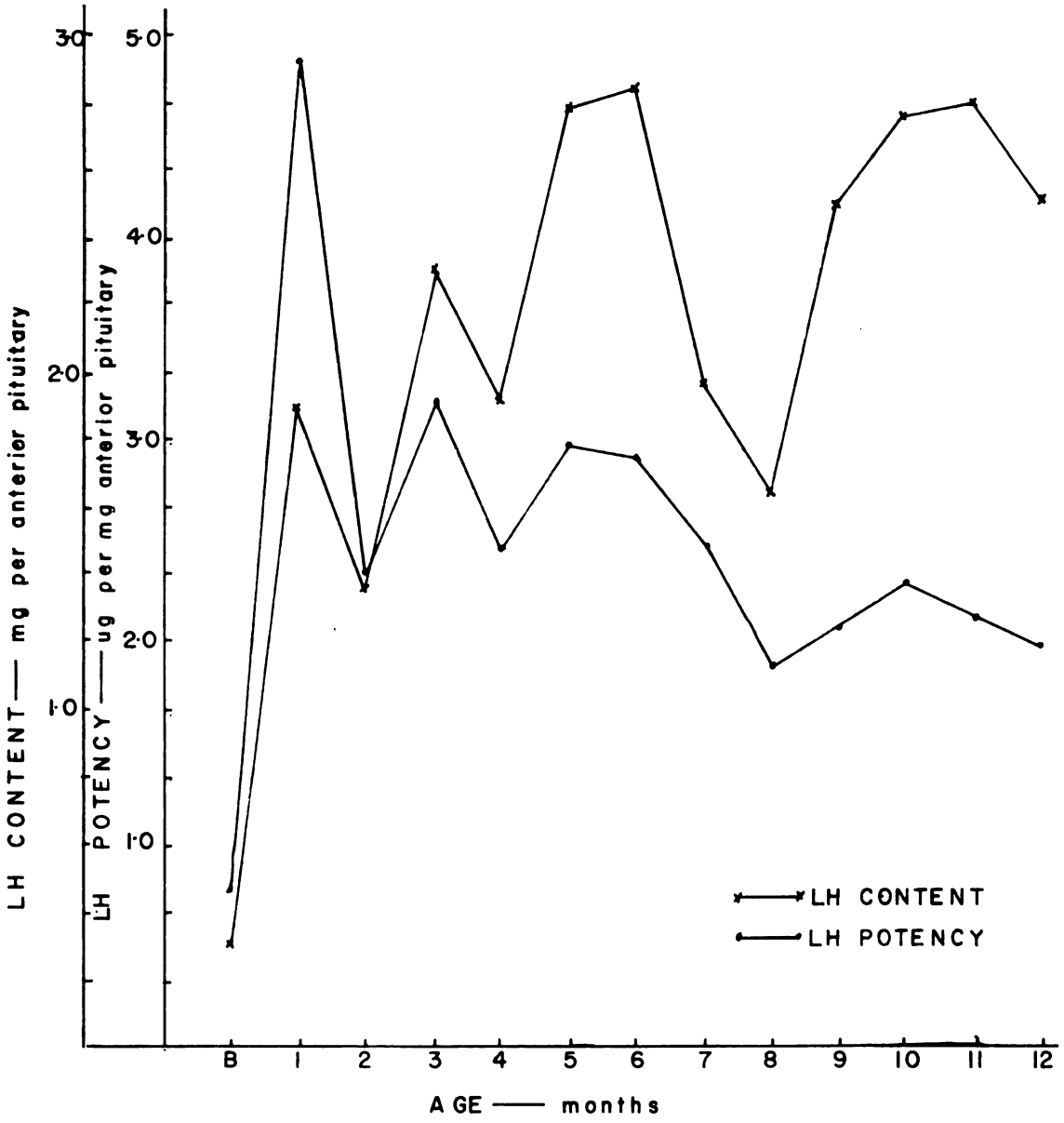

Figure 4.--Changes in pituitary LH content and LH potency in Holstein bulls.

month of age whereas at this age, the bulls possessed their greatest average potency. This is the only age at which the average LH potency for the bulls exceeded that of the heifers of comparable age. A similarity in the data for the bulls and heifers, is that between birth and 9 months of age, both potency curves showed three peaks. These peaks are at 1, 3 and 5 months of age in bulls and 3, 5 and 7 months of age in heifers.

In an attempt to integrate the changes in pituitary weight, LH potency and total LH content, the correlation coefficients between the three parameters were calculated (Fig. 5). The data were considered in three ways--coefficients for the unadjusted data, for data corrected for age differences (within age groups), and for the monthly averages for each parameter (among age groups). Although the coefficients between gland content and the other two parameters are not independent, since total LH content is the product of pituitary potency and weight, the results show that pituitary potency and weight bear different relationships to total pituitary LH. For the data unadjusted for age (Fig. 5,a), the relationships of weight and potency to gland content are similar, but neither is of great predictive value. However, within an age group (Fig. 5,b) potency has a much greater influence on total LH, whereas among age groups (Fig. 5,c), differences in pituitary weight are of greater significance. Pituitary weight and LH potency are quite independent.

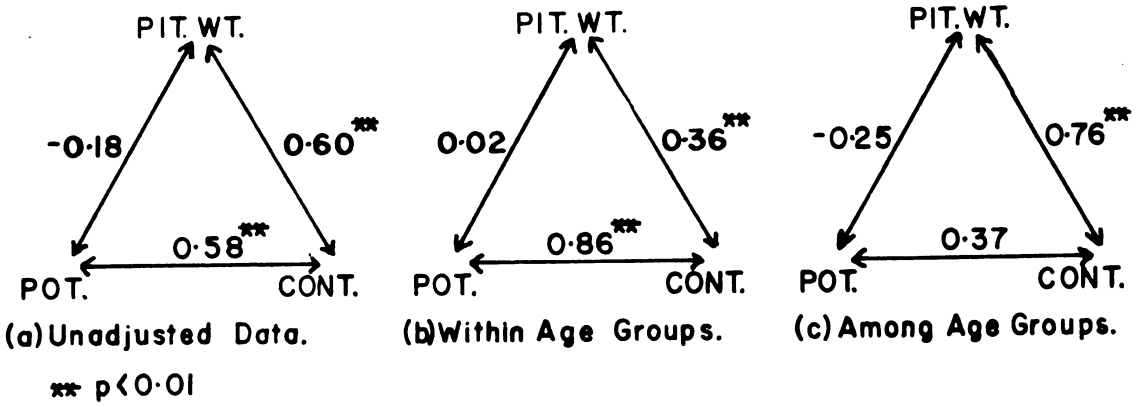

Figure 5.--Correlation coefficients between anterior pituitary weight and pituitary LH potency and LH content.

Some workers present gonadotropic data in terms of ug per gland and others prefer to use ug per mg of gland. For data similar to that from the bulls in this study it would appear that both parameters should be considered because the changes in total content per gland reflect changes in pituitary weight as well as changes in potency.

These differences of opinion regarding the physiological significance of concentration and content can also be found in reports of the plasma gonadotropins. In the case of LH, Armstrong and Greep (1965) showed that bovine LH could be potentiated by administering the hormone is a beeswax-oil vehicle. They hypothesized that this procedure prolonged the release of LH and it was not inactivated as rapidly as when it was injected in saline. However, equine LH could not be potentiated suggesting that it was not inactivated as rapidly as bovine LH in the rat. Parlow and Ward (1961) demonstrated that the half-lives of murine LH, HCG and PMS in mature female rats were 0.28, 4.9 and 26.0 hours, respectively. Although a gonadotropic concentrating mechanism in the gonads has not been demonstrated, Eisenfeld and Axelrod (1966) showed that rats possessed binding sites of limited capacity for H^3-estradiol in the anterior pituitary, uterus, vagina and hypothalamus.

The monthly averages for the concentration of plasma LH and for the total amount of plasma LH per animal are

presented in Table 8. Total plasma LH per animal represents the product of plasma LH concentration and 3.5 percent of the body weight. This value is quoted by Dukes (1955) as the relationship between total plasma volume (in litres) and body weight (in kg). This LH parameter is shown graphically in Fig. 6. The graph shows that the amount of LH in the blood plasma almost triples between 2 and 3 months of age. There is a plateau from 4 to 6 months of age and then a further dramatic rise to 10 months of age. These data indicate that puberty or reproductive development may be biphasic. There is an initial developmental phase lasting from 3 to 6 months of age and a secondary phase lasting from 6 to 9 or 10 months. The absolute increases in total plasma LH are greatest during the second phase.

The values for the plasma concentrations of LH (Table 8) should be regarded as relative rather than absolute because the data in Table 9 show that the recovery rate of LH was only 25 percent. These recovery rate data were derived by pooling plasma from many animals until there was sufficient for nine assays. No NIH-LH was added to three samples, 10 ug LH per liter to another three samples and 100 ug LH per liter to the remaining three samples. Then the LH was extracted and assayed as previously outlined with one sample from each treatment being extracted simultaneously. This is the first time that an estimate of the efficiency of this LH extraction method,

TABLE 8.--Average plasma concentrations and total plasma content of luteinizing hormone and the average ratio between total plasma content and total pituitary content of luteinizing hormone.

Age	LH		
	Plasma Concentration	Plasma Content	Plasma[c] Pit. Ratio
(months)	(ug/l)[a]	(ug/animal)[b]	
Birth	0.48[d]	0.59[d]	1.97
1	0.41[d]	0.63[d]	0.33
2	0.17 ± 0.03	0.43 ± 0.08	0.34
3	0.34 ± 0.09	1.17 ± 0.36	0.49
4	0.35 ± 0.09	1.72 ± 0.45	0.88
5	0.29 ± 0.08	1.51 ± 0.41	0.53
6	0.24 ± 0.06	1.50 ± 0.43	0.61
7	0.30 ± 0.09	2.14 ± 0.59	1.50
8	0.42 ± 0.10	3.32 ± 0.77	2.38
9	0.42 ± 0.12	3.90 ± 1.22	1.51
10	0.50 ± 0.14	5.08 ± 1.48	2.33
11	0.38 ± 0.10	4.31 ± 1.07	1.87
12	0.47 ± 0.11	5.47 ± 1.18	2.22

[a]ug NIH-LH-B3 equivalent per liter.

[b]ug LH per liter x body wt. (kg) x 0.035.

[c]ug plasma LH per animal ÷ mg pituitary LH per animal.

[d]Estimates derived from pooled samples.

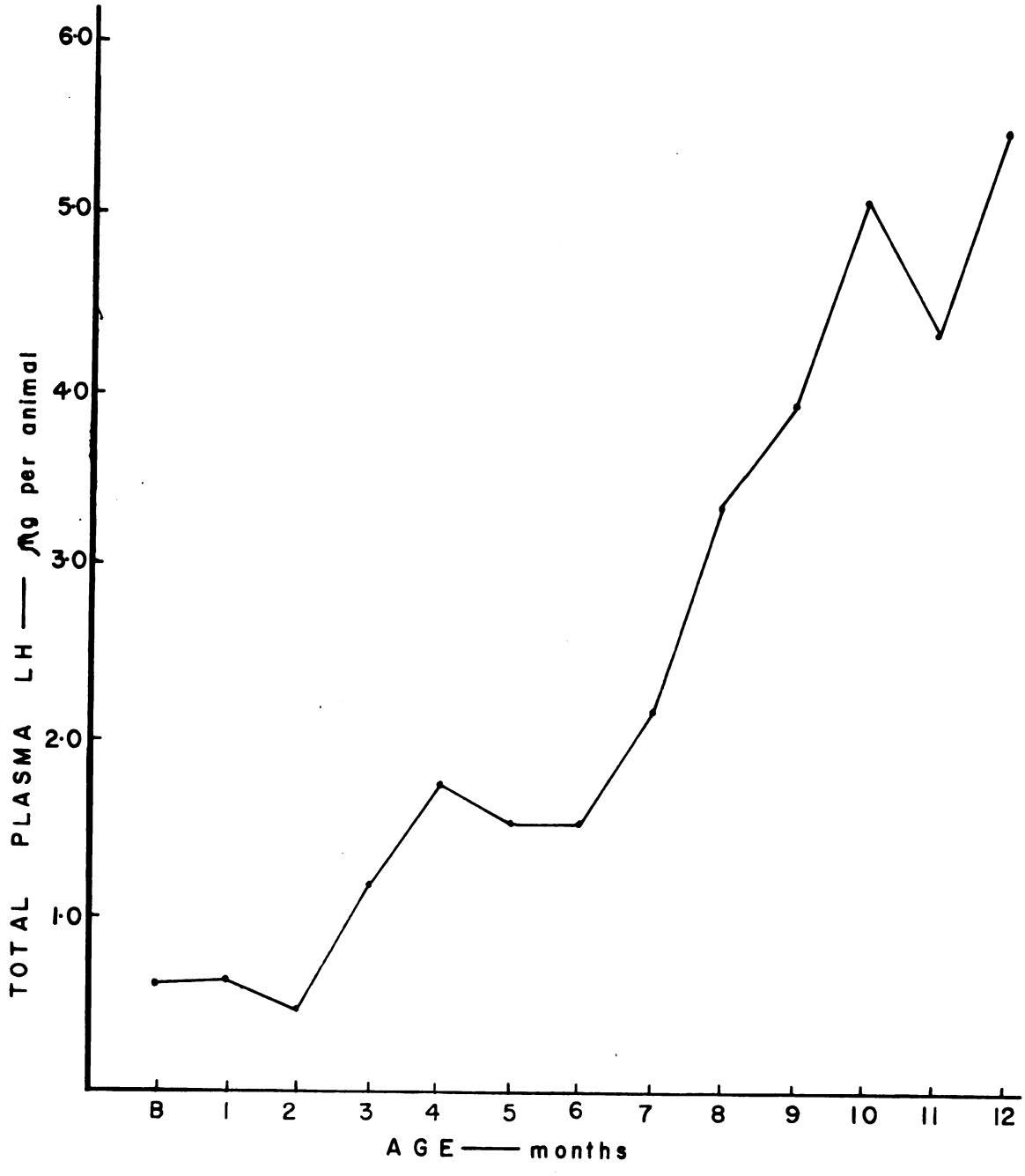

Figure 6.--Changes in plasma LH content per animal to 12 months of age.

which was developed by Anderson and McShan (1966), has been objectively determined. Because they did not determine

TABLE 9.--The recovery rate of NIH-LH-B3 when added to bovine blood plasma.

Replicate	NIH-LH-B3 Added per Liter of Plasma				
	Assayed Potency[a]			Percent Recovery	
	0	10 ug	100 ug	10 ug	100 ug
1	0.48	3.31	27.73	28.3	27.3
2	0.33	2.95	28.30	26.2	28.0
3	0.43	2.18	23.24	17.5	22.8
Mean	0.41	2.81	26.42	24.0	26.0

[a]ug NIH-LH-B3 equivalent per liter.

their efficiency of extraction from the plasma of lactating dairy cows, comparisons of their LH data with those from this study in bulls may not be justified. However, Ramirez and McCann (1963) could not detect LH in the plasma of intact immature male rats and the levels in mature male rats were minimal.

Another correction which would have to be made involves the loss of LH in (or on) the blood cells during centrifugation of the blood. The extent of this loss is not known. If a correction factor of 4.0 is used based on the data in Table 9, and it is assumed that the half-life

of LH in the bull is similar to that in the rat (0.30 hours), calculations show that the pituitary of a 2 month old bull would release 52.0 ug LH per day. The comparable figures for 4, 8 and 10 month old bulls are 208, 399 and 610 ug LH per animal per day, respectively.

The correlation coefficients between the LH parameters in the blood and in the pituitary were small (-0.13 to + 0.10). However, Fig. 7 relates the rate of release of LH as measured by total plasma LH to the degree of storage as measured by the total pituitary content of LH. This graph shows a precipitous decline from birth to 1 month of age. Together with the data on the plasma LH concentration, this result indicates that at birth the blood plasma contains proportionately higher values of LH-like gonadotropins. These gonadotropins may be released from the animal's own pituitary due to stimulation by maternal releasing factors derived from the maternal hypothalamus or the placenta. The LH-like response may also be due to hormones other than LH because the extraction procedure does not distinguish between LH, PMS and HCG. Similar hormones of placental origin may not yet have been eliminated from the blood stream of the day-old calf.

Further consideration of Fig. 7 indicates that the release to storage ratio peaks suddenly at 4 months of age and again rises rapidly from 6 to 8 months of age. Thereafter the changes are erratic, but from 7 months of

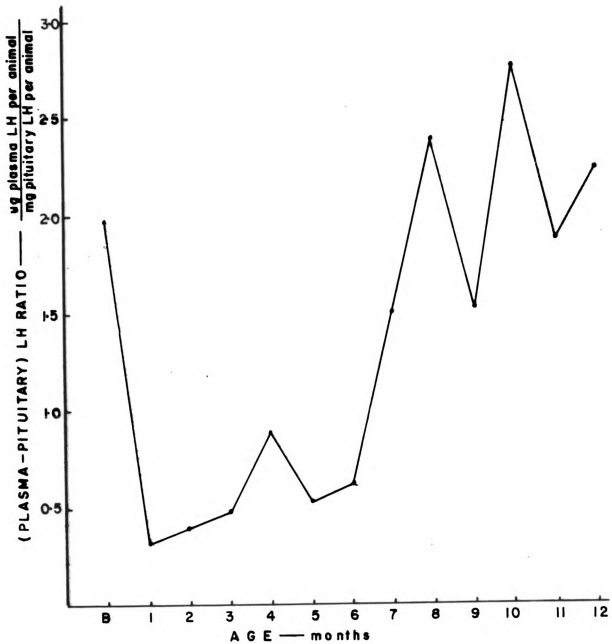

Figure 7.--Changes in the ratio between total plasma LH and total pituitary LH.

age the ratio is always greater than 1.5. The total amount of plasma LH increased rapidly from 6 to 10 months of age (Fig. 6) but the data on the release to storage ratio indicates that the most dramatic increase in release relative to storage occurs between 6 and 8 months of age.

Taleisnik and McCann (1960) showed that pharmacological dose levels of vasopressin could reduce the ovarian concentration of ascorbic acid when injected into pseudopregnant rats. The possibility remained that the acetone extraction procedure used in the present research could be concentrating vasopressin as well as LH. To determine whether vasopressin was influencing apparent LH responses, two levels of "Pitressin" (Parke Davis) were added to composite plasma samples which were extracted and compared to a similar plasma sample to which no vasopressin was added. The results are presented in Table 10 and show that the effect of adding vasopressin to the plasma was inconsistent. Since a ten-fold increase in the amount of added vasopressin did not increase the apparent potency of LH, the estimate of 0.61 with 10 IU Pitressin per liter is considered to be due to sampling error.

In summary, the two pituitary LH parameters did not reveal any dramatic changes which could be interpreted to mean that the period of puberty commenced or was terminated at any particular age. The data for total plasma

TABLE 10.--The effect of adding "Pitressin" to blood plasma on subsequent luteinizing hormone determinations.

Amount of Added Pitressin	"LH" Potency[a]	Difference From Control[b]
0	0.38	--
10 IU per liter	0.61	0.23
100 IU per liter	0.46	0.08

[a]ug NIH-LH-B3 equivalent per liter.

[b]Difference from control = increase in potency due to added "Pitressin."

LH content, however, indicate that the period of puberty is biphasic with an initial rise in plasma LH at 3 months followed by a plateauing to 6 months and then another dramatic increase to 10 months of age. In terms of the rate of LH release to net LH storage (the ratio between plasma content and pituitary content) trends are similar to total plasma LH except that a "mature" ratio is attained by 8 months of age.

(c) Follicle Stimulating Hormone (FSH).--The data for the parameters involving pituitary FSH are presented in Table 11 with the comparative data obtained from heifers by Desjardins (1966). As can be seen in Fig. 8, total pituitary FSH and pituitary FSH potencies show different

TABLE 11.--Average pituitary potency and content of FSH and the LH:FSH ratio in bulls from birth to one year of age and comparable potency values in heifers.

Age	Bulls			Heifers[a]
	Potency	Content	LH:FSH	Potency
(months)	(ug FSH/mg)[b]	(ug FSH/pit)		(ug FSH/mg)[c]
Birth	0.18	70.2	4.2	1.67
1	0.17	66.3	28.7	2.68
2	0.24	134.4	9.8	1.06
3	0.19	138.7	16.6	0.83
4	0.16	128.0	15.3	0.98
5	0.19	176.7	15.6	0.94
6	0.19	182.4	15.2	0.89
7	0.19	152.0	13.0	0.92
8	0.15	130.5	12.5	1.01
9	0.08	99.2	25.6	0.93
10	0.12	142.8	19.0	0.94
11	0.07	93.1	30.3	0.81
12	0.09	114.3	21.8	0.79

[a]Data from Desjardins (1966).

[b]ug NIH-FSH-S3 equivalent per mg fresh anterior pituitary.

[c]ug NIH-FSH-S2 equivalent per mg fresh anterior pituitary.

NOTE: Relative potency of S2 = 0.87; S3 = 1.10.

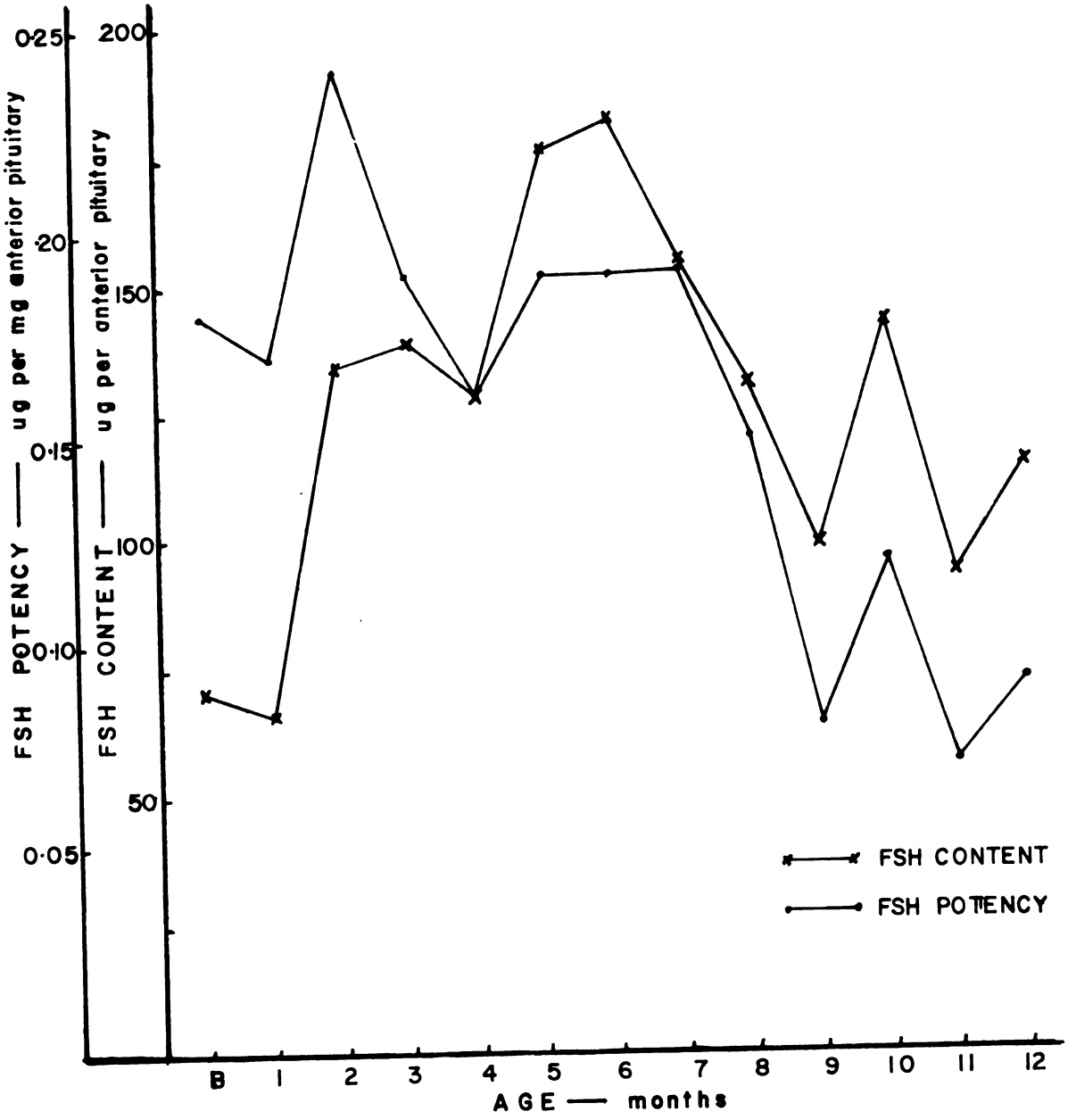

Figure 8.--Changes in pituitary FSH content and FSH potency in Holstein bulls.

age trends. As indicated in Materials and Methods, FSH potency was not estimated for each pituitary, but rather on composite within age group samples. Consequently, the figure for total FSH content must be regarded only as an approximation, as it represents the product of the potency estimate from the pooled pituitaries and the appropriate average pituitary weight. The correlation coefficients between pituitary potency, content and weight are presented in Fig. 9. This diagram is comparable to Fig. 5(c) for LH. However, whereas total pituitary LH is significantly correlated with total pituitary weight, neither FSH potency nor pituitary weight is significantly correlated with total pituitary FSH. The negative correlation between average pituitary weight and FSH potency explains why the age changes in total pituitary FSH are not as proportionately great as the comparable figures for LH. Pituitary weight increases with age but an associated decline in FSH potency means that the pituitary FSH content remains relatively constant.

The FSH potency is greatest at 2 months of age but shows little change between 3 and 7 months of age. Thereafter the potency declines. However, the pituitary FSH content increases irregularly to 6 months of age. If there is an inverse relationship between pituitary and plasma contents of FSH, then the decline in pituitary FSH content from 6 to 9 months of age would be associated with

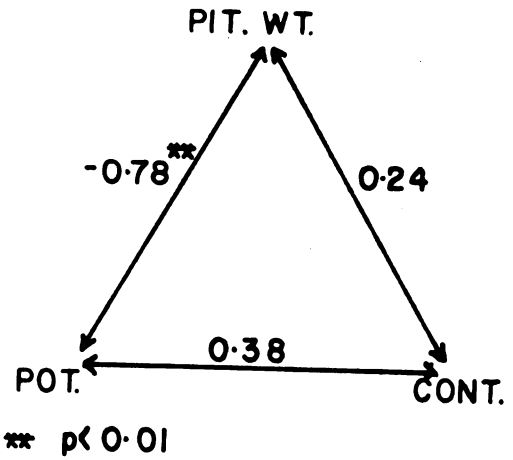

Figure 9.--Correlation coefficients between monthly averages for pituitary weight and pituitary FSH potency and FSH content.

elevated blood levels of FSH. These increasing blood levels of FSH would occur at the same age as the measured increases in the plasma content of LH (Fig. 10). The similarity in the changes in potency and content of LH and FSH between 3 and 8 months of age is reflected by the constancy of the LH/FSH ratio (Fig. 10). Before and after this age range the larger fluctuations in LH mask the minor fluctuations in FSH.

A comparison of the FSH potency data for the bulls with that for heifers clearly shows that the heifers have much higher levels of pituitary FSH. Since the heifers also had heavier anterior pituitary glands, differences in FSH content would be even greater. The difference in potencies cannot be fully explained by the difference in the relative potencies of the two standard preparations which were used. Desjardins (1966) suggested that FSH potency in heifers exhibited a slight decline after 10 months of age. This decline must be quite dramatic between 12 and 15 months of age as Hackett and Hafs (unpublished data) showed that, during the estrous cycle in 15 to 16 month old heifers, the FSH potency fluctuated between 0.09 and 0.29 ug NIH-FSH-S2 equivalent per mg of fresh pituitary.

It would appear therefore that during reproductive development in the heifer the potency of FSH is greatly elevated and subsequently declines. By contrast, the FSH potency in bull pituitaries is not elevated during

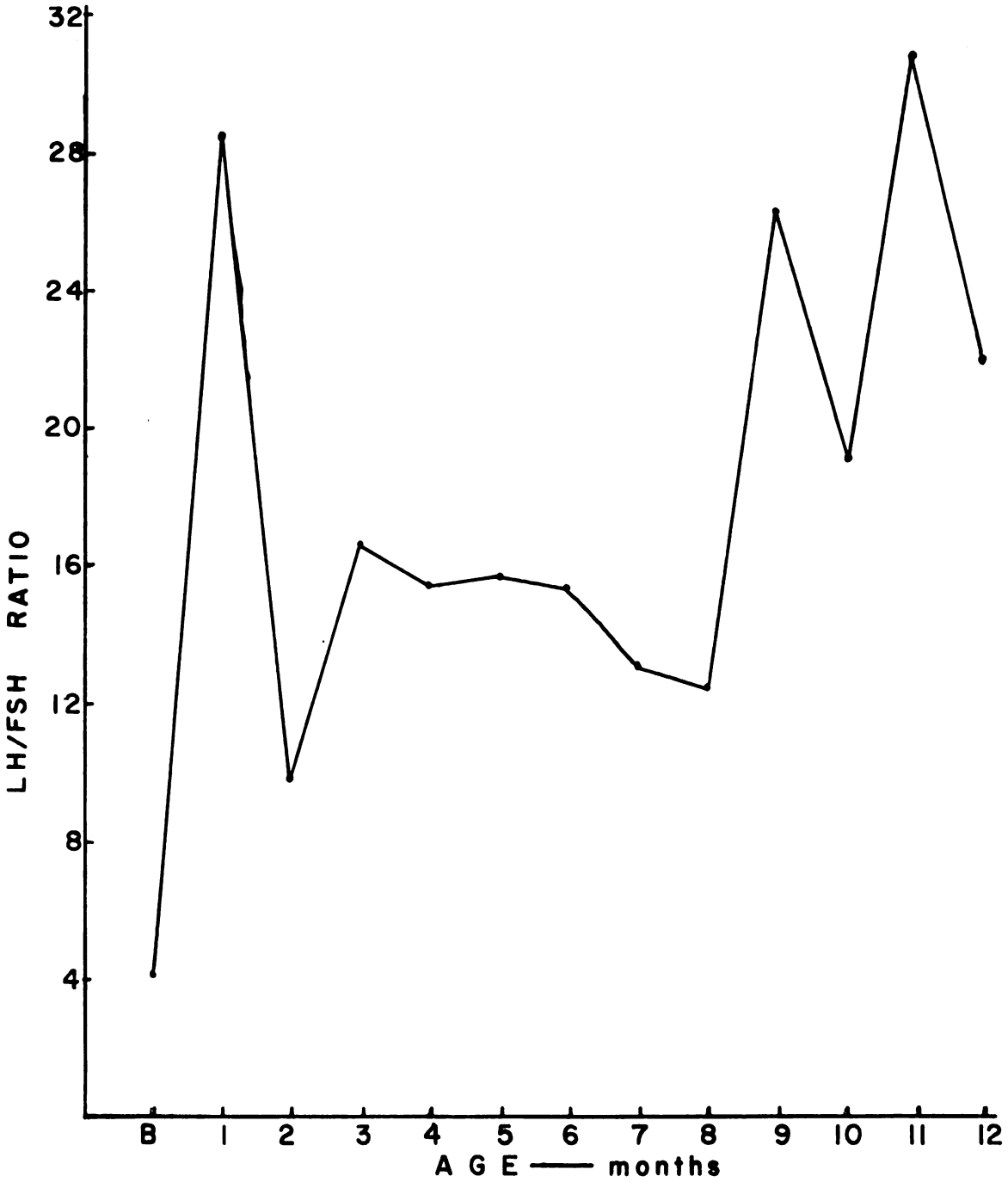

Figure 10.--Changes in pituitary LH : FSH ratio to 12 months of age.

reproductive development. Consequently the bovine male pituitary has a much lower FSH potency than the female during reproductive development, but probably possesses potency similar to that of the heifer by 15 months of age.

The heifer pituitary also has a greater concentration of LH than the pituitary of a similarly aged bull. However, the proportional difference was not as great as that for FSH. Consequently the LH:FSH ratio is higher in bulls than in heifers of comparable age.

The data in both bulls and heifers show that FSH-potency changes with advancing age in the bovine pituitary are different from the maturation changes in the rat pituitary. Hoogstra and Paesi (1955) reported that the potency of FSH in adult male rat pituitaries was five times greater than the FSH potency in female rat pituitaries. By contrast, at 12 months of age the FSH potency of the heifer's pituitary is almost nine times greater than the FSH potency of the bull's pituitary. Although 12-month old Holsteins are not mature animals, both sexes can fulfill their appropriate reproductive roles and cannot be regarded as immature. Like the rat, the immature heifer pituitary does have a higher concentration of FSH than the immature bull pituitary but the sexual difference in Holsteins is much greater than in rats.

(d) Growth Hormone (GH).--Similarly to FSH, the estimates of GH potency (Table 12 and Fig. 11) are based on composite within age group samples, and the average GH content per pituitary is only an approximation because it represents the product of the composite potency estimate and the

TABLE 12.--Changes in the pituitary potency and pituitary content of growth hormone.

Age	GH Potency	GH Content
(months)	(ug GH/mg)[a]	(mg GH/pituitary)
Birth	23.33	9.10
1	16.09	6.28
2	56.66	31.73
3	123.64	90.26
4	126.79	101.43
5	94.92	88.28
6	57.01	54.73
7	44.33	35.46
8	43.50	37.85
9	46.61	57.80
10	27.65	32.90
11	36.70	48.81
12	23.62	30.00

[a]ug NIH-GH-B9 equivalent per mg fresh pituitary.

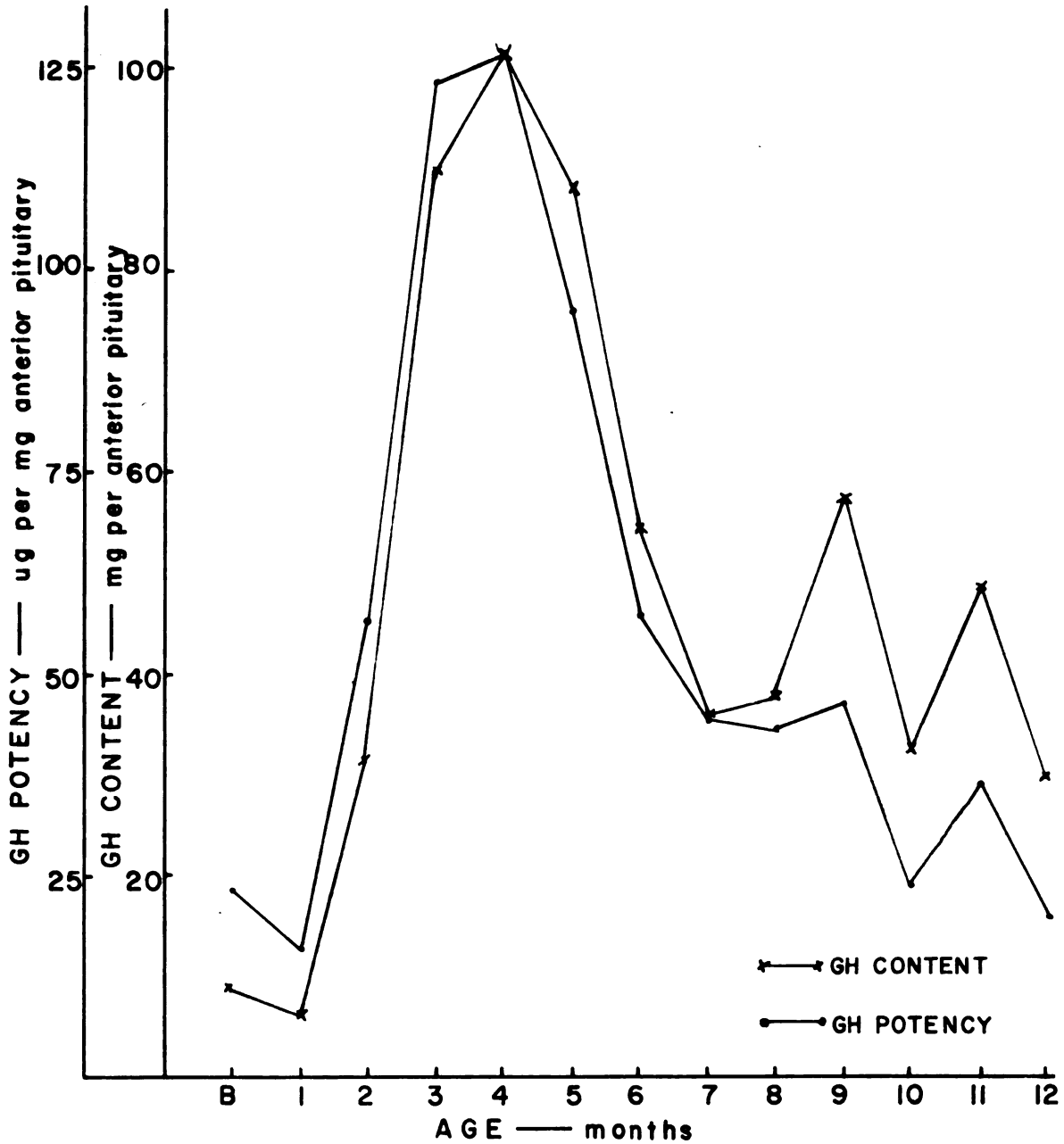

Figure 11.--Changes in pituitary GH content and GH potency in Holstein bulls.

comparable average pituitary weight. The curves for the total GH content and GH potency are similar, except that a regular decline in potency is reflected as an irregular decline in total GH content.

It is unlikely that the TSH content of the pituitary extracts influenced the GH potency estimates because TSH was added to the standard GH preparations so that they contained 1 ug TSH per ug GH. Schooley et al., (1966) recently verified the reports of earlier workers who showed that TSH can augment the tibial response in hypophysectomized rats but this augmentation does plateau. Meites (personal communication) considered that the TSH:GH ratio (1:1) used as the standard hormone preparations in this experiment would adequately compensate for the lack of TSH in a pure GH standard preparation. Nonetheless, TSH levels were not measured in the pituitary extracts and they may have varied among ages and fallen below the "plateau level of augmentation." Reece and Turner (1937) reported that TSH potency changes were not very great in dairy cattle of either sex from birth to 12 months of age. If the TSH potencies were in fact below the "plateau level," then comparison with a GH standard preparation containing TSH would depress the GH potency estimates. On the other hand, if TSH were not added to the GH standard preparations, all the potency estimates would be elevated.

Although the pituitary potency and pituitary content of GH increase rapidly from 1 to 4 months of age, and

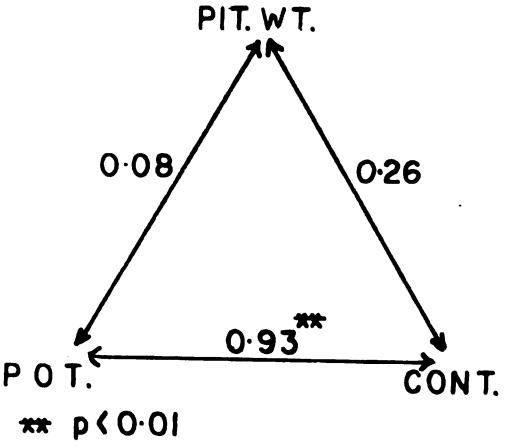

Figure 12.--Correlation coefficients between monthly averages for pituitary weight and pituitary GH potency and GH content.

then decline, these fluctuations are not reflected in growth rate. Neither do these changes appear to be related to the changes in either of the gonadotropins. Since it is known that GH has a metabolic role in addition to a growth function, it could be speculated that dietary changes influence GH potency and content. The bulls were fed on whole milk to 4 months of age and were then weaned but continued to receive a high protein grain supplement.

A comparison of this data for bulls with the results obtained by Armstrong and Hansel (1956) in heifers shows that the GH potency is much greater in bulls than in heifers. At 7 to 8 months of age, they estimated the GH potency at 11.66 mg GH per gram of fresh anterior pituitary tissue. In bulls of comparable age the average potency is over 43 mg and in addition the standard used in the present study was probably of greater purity and potency than that used by Armstrong and Hansel (1956). Whereas the GH potency in heifers declined from 8 months of age, the decline in the bull GH potency commences after 4 months of age.

The correlation coefficients diagrammatically summarized in Fig. 12 show that the variations in the pituitary GH content are primarily due to changes in pituitary GH potency. This result shows that the age variation in the pituitary content of LH, FSH and GH are influenced by the variation of different factors. In the case of LH it is

pituitary weight (r = 0.76) and for GH it is pituitary potency (r = 0.93). The pituitary content of FSH does not show the same large variations as the other two hormones because of the negative correlation between pituitary weight and pituitary potency (r = -0.78).

C. CHANGES IN THE TESTES AND THE EXCURRENT DUCTS

(a) Testicular Growth.--The parameters used for measuring testis growth were weight, volume, length from pole to pole and the mid-point diameter (Table 13). In contrast to date obtained by Abdel-Raouf (1960), there was no consistent weight difference between left and right testes. In Swedish bulls, the right testis was consistently heavier. Data presented by Bratton et al., (1959) shows a similar difference may occur in young Holstein bulls. However, Almquist and Amann (1961) found that within-bull testis-weight variation was very small and the left testes of the 55 bulls (mostly Holsteins) averaged 8.1 g heavier. In the present study, the average testis weight for each bull was calculated. The other three criteria were only measured on left testes.

Testicular weight increases with age, following a quadratic growth curve from birth to 9 months of age (Fig. 13). Thereafter, the rate of increase declines. Changes in testicular volume are very similar to those for weight. However, the testicular mid-point diameter and pole-to-pole length increase linearly from birth to 9

months of age. Subsequent increases are small. Nine months appears to be the age at which the rate of change in each of these four parameters declines, indicating that the period of most rapid development has ended.

TABLE 13.--Changes in the weight, volume, length and diameter of the testis.

Age	Testis Weight[a]	Testis Volume	Testis Length	Testis Diameter
(months)	(g)	(ml)	(cm)	(cm)
Birth	2.47	5.3	2.6	1.3
1	3.57	4.8	2.8	1.4
2	7.68	8.4	3.7	1.8
3	12.33	12.1	4.2	2.4
4	22.87	22.4	5.6	2.8
5	34.16	32.2	5.9	3.2
6	47.21	44.9	6.5	3.7
7	64.34	79.2	6.9	4.0
8	102.97	93.0	8.3	4.8
9	165.52	161.9	9.8	5.5
10	156.90	151.2	9.5	5.5
11	172.76	164.2	10.3	5.7
12	203.56	197.0	10.5	5.8

[a]Calculated from the mean testis weight for each bull.

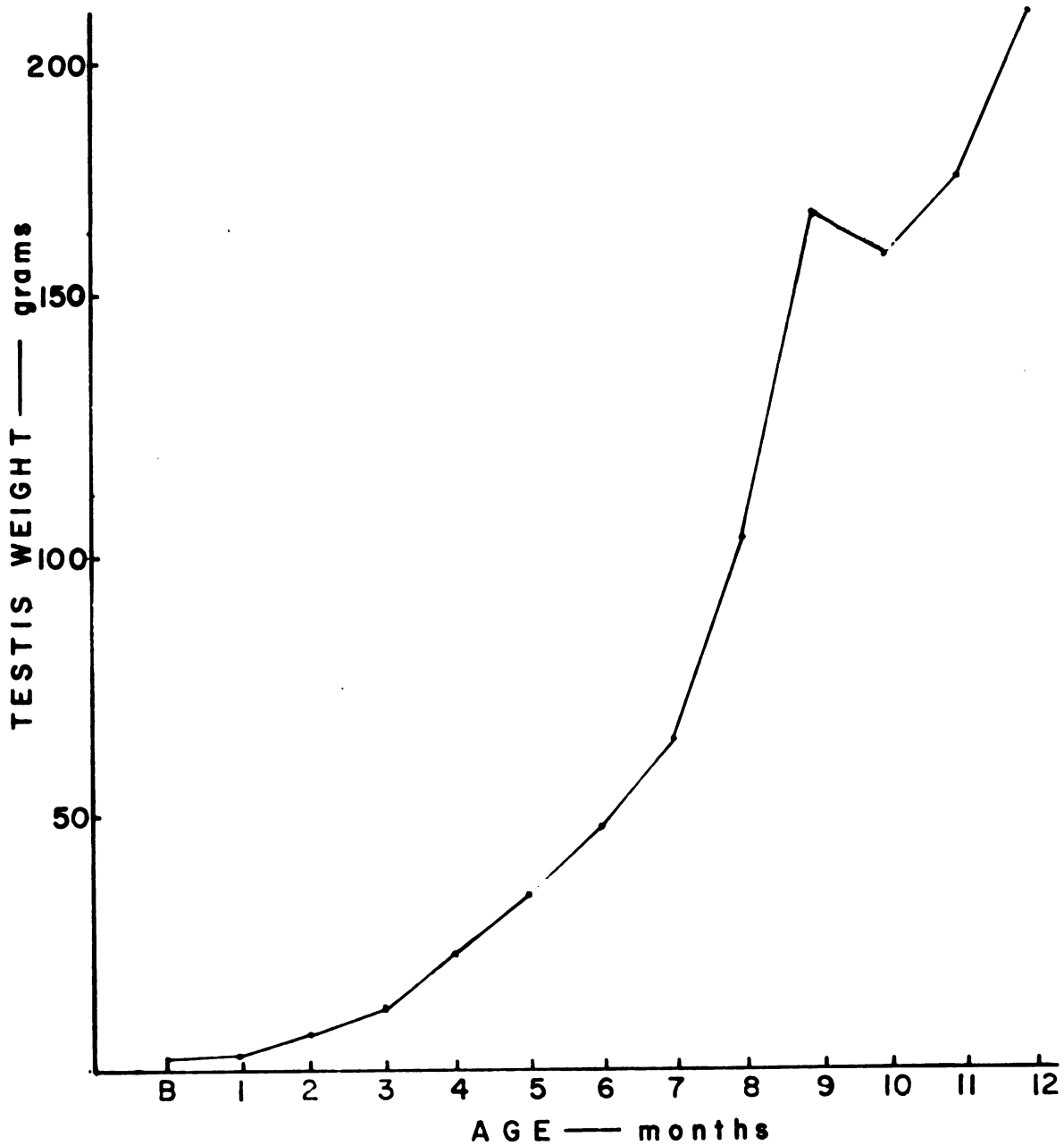

Figure 13.--Changes in testis weight in Holstein bulls to 12 months of age.

Abdel-Raouf (1960) concluded that testes of Swedish Red-and-White bulls become more oval in shape with advancing age because of the greater increase in breadth than in length. However, the change in testicular length in Holstein bulls from birth to 9 months represents a proportional increase of 3.8 and the comparable change in breadth is only slightly greater (4.2).

(b) Testicular Nucleic Acids.--The average testicular contents of RNA and DNA increase rapidly from 3 to 12 months of age (Table 14) with the proportional increase in RNA being the greater. However, the standard errors also increase. The standard error of the average RNA content at 3 months is 4.41 and at 9 months is 136.57. (Appendix III.) Because of this increase in variation, the decline in DNA and RNA at 10 months of age may merely reflect sample variation. By contrast, the variation in the nucleic acid concentrations declines with advancing age.

Since testicular weight exhibits a reduced rate of increase from 9-12 months of age, but nucleic acid contents do not, the nucleic acid concentrations increase slightly at 11 and 12 months of age. These concentrational changes (Fig. 14) show that the testicular DNA concentration declines at a diminishing rate from birth to 10 months of age. Similarly to testicular weight and volume, the increases in testicular DNA and RNA contents follow a quadratic curve.

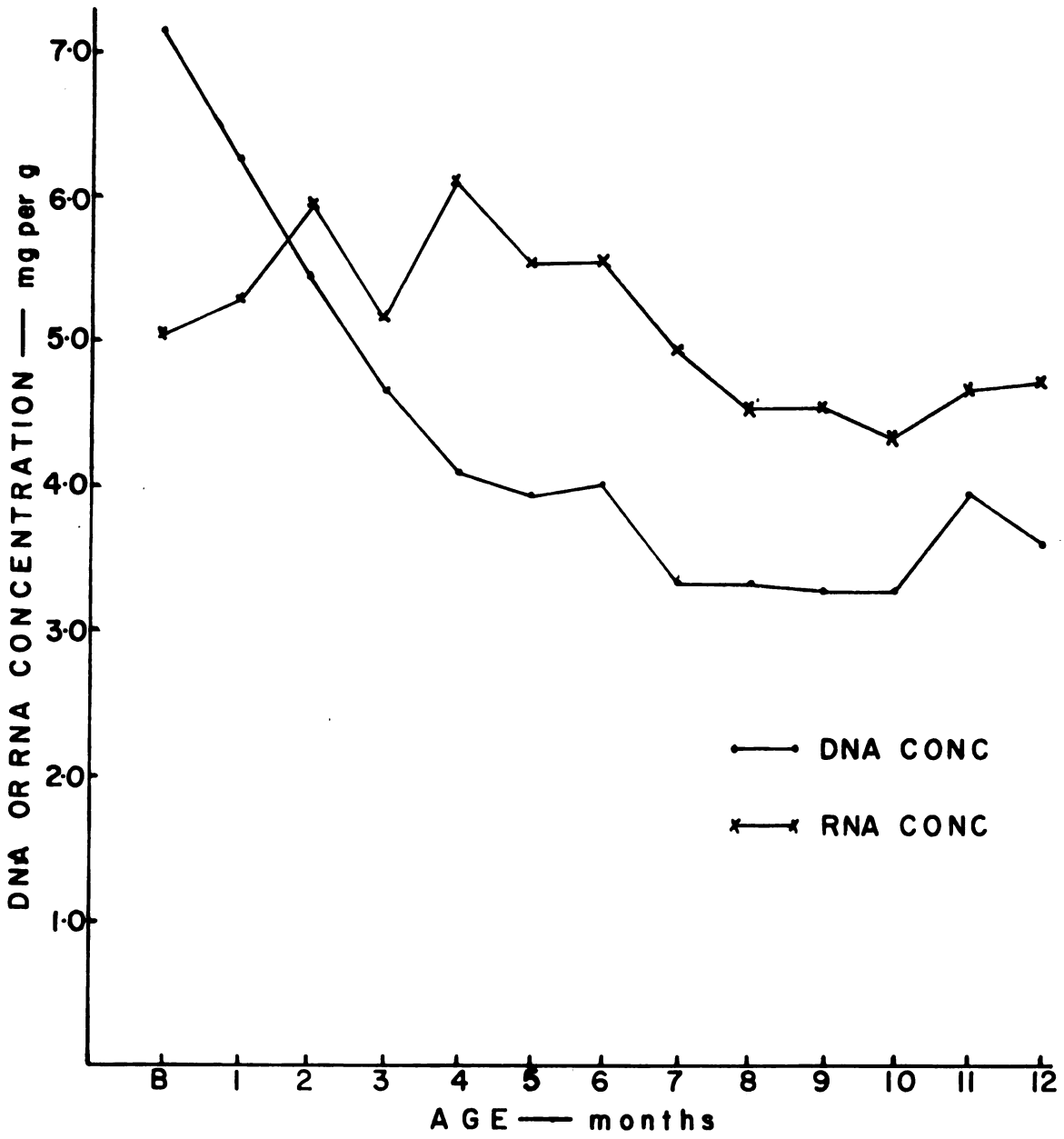

Figure 14.--Changes in the concentrations of testicular DNA and RNA.

The decline in DNA concentration in the bull testis with advancing age follows a curve which is very similar to the decline in testicular DNA concentration in the rat (Fujii and Koyama, 1962; and Desjardins, Macmillan and Hafs, unpublished data). This decline in both species is partly due to the increase in the average diameter of seminiferous tubules. At birth, the tubules in bulls are solid sex chords but the tubules increase for several months before a lumen forms (Abdel-Raouf, 1960). This increase in diameter is linear from 1 month to 10 months of age whereas the decline in DNA concentration is a quadratic curve. Evidently the onset of spermatogenesis partially compensates for the loss of DNA concentration due to lumen formation. As the sperm numbers increase in the tubules at an increasing rate from 5 months of age, the rate of decline in DNA concentration is reduced and is finally reversed.

Despite the increase in the diameter of the seminiferous tubules, testicular RNA concentration continues to increase to a maximum of 6.13 mg per gram at 4 months of age. Thereafter there is a slow decline to 4.34 mg per gram at 10 months of age. Since spermatogenesis is associated with a loss of cytoplasm by the sperm cell, the increase in total RNA and slight decline in RNA concentration from 4 months of age is probably due to increasing numbers of Leydig cells and increasing numbers and activity of the Sertoli cells. In rats, Fujii and

Koyami (1962) found that testicular RNA concentration declined with increasing age but the decline was at a slower rate than for DNA concentration.

In the case of the developing or adult testis, the RNA:DNA ratio is not necessarily indicative of protein synthetic activity per cell, since spermatozoan DNA would reduce the ratio. However, since RNA concentration increases to 4 months of age and the DNA concentration declines, the testicular RNA:DNA ratio increases rapidly to 4 months of age but then declines slowly.

TABLE 14.--Changes in testicular nucleic acid concentration and content and the RNA:DNA ratio.

Age	DNA Conc.[a]	RNA Conc.[a]	Total DNA	Total RNA	RNA/DNA
(months)	(mg/g)	(mg/g)	(mg/testis)	(mg/testis)	
Birth	7.18	5.05	13.64	9.71	0.70
1	6.26	5.32	17.28	15.39	0.90
2	5.43	5.94	34.93	37.80	1.11
3	4.64	5.19	43.40	49.26	1.13
4	4.09	6.13	79.72	120.35	1.54
5	3.95	5.56	111.23	157.19	1.43
6	4.01	5.55	168.12	235.30	1.39
7	3.35	4.94	194.59	290.15	1.47
8	3.34	4.56	302.30	414.56	1.38
9	3.28	4.59	510.86	722.27	1.40
10	3.27	4.34	448.40	599.16	1.33
11	3.95	4.67	607.15	732.70	1.27
12	3.60	4.72	666.83	873.30	1.31

[a]Concentration

(c) Seminiferous Tubule Changes.--At birth all the seminiferous tubules are solid chords (Table 15). These chords increase in diameter, and lumen formation is apparent in some bulls at 3 months of age. From birth to 8 months of age the seminiferous tubules are usually circular in outline but at later ages, when the tubules are close together, the outline of many tubules is distorted. The average tubule diameter increases linearly from 1 to 10 months of age (Fig. 15). During this period there is a four-fold increase in diameter at an average rate of 16.9 microns per month.

These data from Holstein bulls are similar to that obtained by Abdel-Raouf (1960) for Swedish Red-and-White bulls. In this latter breed the rate of increase in tubule diameter showed a sudden decline at 36 weeks of age, whereas a decline in diameter occurs in the Holstein at 10 months. Evidently tubule diameter continues to increase at an average rate of about 5 microns per month from 12 to 29 months of age as Amann (1962) reported that the average tubule diameter was 254 microns in 29 month old Holstein bulls. The comparable figure in mature bulls was 271 microns.

Mature spermatids were detected in one 6 month old bull and in all bulls which were 8 months of age or older. Since the histological parameters in each bull only involved one small sample from one testis, the results do

TABLE 15.--Changes in some characteristics of seminiferous tubules from birth to 12 months of age.

Age	Tubule Diameter	Degree of Tubule Development		
		All Solid Tubules	Lumen Present	Sperm Present
(months)	(microns)	---------- (no. of bulls) ----------		
Birth	42.8	5	0	0
1	45.5	5	0	0
2	57.9	5	0	0
3	65.1	4	1	0
4	82.6	2	3	0
5	96.3	0	5	0
6	130.2	0	4	1
7	128.6	0	3	2
8	157.6	0	0	5
9	181.9	0	0	5
10	197.2	0	0	5
11	193.9	0	0	5
12	172.4	0	0	5

not preclude the possibility that spermatozoa may have been present in any given testis and not detected in the stained section. Knudsen (1954) found large variations in the spermatogenic activity in different tubules within any one testis in young bulls. The accompanying photomicrographs (Fig. 16) show the degree of variation in the

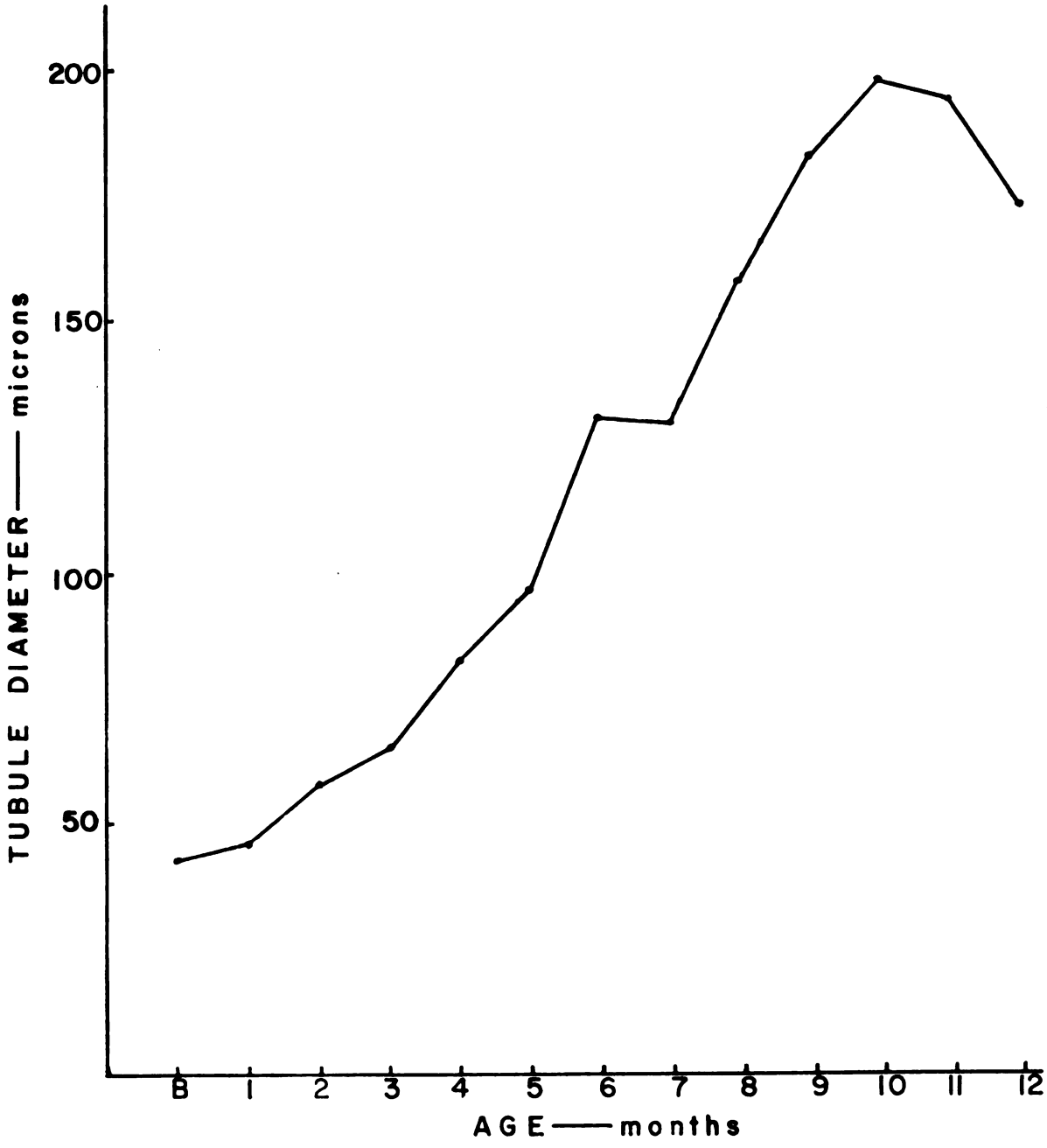

Figure 15.--Changes in the average diameters of the seminiferous tubules.

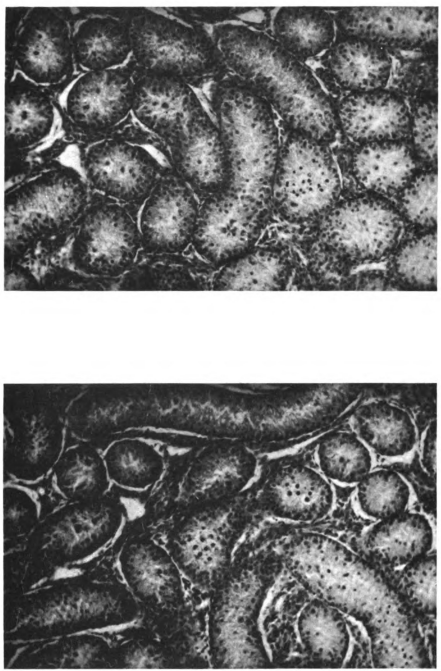

Figure 16.--Photomicrographs of a 5 month-old bull (No. 161) showing different stages of spermatogenesis in different tubules (x150).

spermatogenic activity within the tubules of a testis from a 5 month old Holstein bull included in the present study. Consequently, the examination of testicular homogenates appears to be a better method for detecting the numbers of spermatozoa and terminal-stage spermatids in the testes of young bulls.

(d) Changes in the Excurrent Ducts.--The ampullae and ductus deferentia are often classified as accessory or secondary sex organs. Since estimates of extra-gonadal sperm reserves include both segments, they are considered here with the epididymides as excurrent ducts.

The weight of the epididymis increases with advancing age following a curve of similar shape to that for testicular weight, with the exception that the epididymal curve is continuous to 12 months of age (Table 16). The increase in weight is largely due to weight changes in the caput epididymidis which shows a 40-fold increase from birth to 12 months of age. Comparable proportional weight changes in the corpus and cauda epididymides are 20-fold and 26-fold increases, respectively. The shape of the epididymal growth curve is similar in Holstein and Swedish Red-and-White bulls (Abdel-Raouf, 1960), but the weight of the epididymides is consistently less to 12 months of age in Holsteins.

TABLE 16.--Changes in the weight of the epididymis and the caput, corpus and cauda segments.

Age	Epididymal Weight[a]	Caput Epididymidal Weight[b]	Corpus Epididymidal Weight[b]	Cauda Epididymidal Weight[b]
(months)	----------	---------- g ----------		----------
Birth	0.54	0.24	0.12	0.17
1	0.55	0.21	0.11	0.19
2	1.36	0.54	0.23	0.52
3	1.82	0.68	0.32	0.63
4	3.00	1.30	0.64	1.09
5	3.80	1.29	0.52	1.45
6	4.96	2.21	1.02	1.65
7	5.17	2.57	1.14	1.59
8	7.89	3.58	1.68	2.44
9	11.35	6.47	1.23	3.57
10	11.77	5.95	2.46	3.21
11	13.87	7.38	1.80	4.23
12	17.12	9.64	2.48	4.47

[a]Calculated from the mean epididymidal weight for each bull.

[b]Calculated from the weights of the left epididymides.

The tubule diameters and epithelial cell heights in the three segments of the epididymis (Table 17) each increase irregularly from birth to 11 months of age. The rate of increase in epithelial cell height in the cauda

TABLE 17.--Changes in the tubule diameters and epithelial cell heights of the caput, corpus and cauda epididymides.

Age	Tubule Diameter			Epithelial Cell Height		
	Caput	Corpus	Cauda	Caput	Corpus	Cauda
(months)	---------- (microns) ----------					
Birth	103.1	78.8	96.1	13.9	17.8	24.5
1	81.0	78.7	106.8	17.9	18.0	25.5
2	108.5	127.1	204.3	24.7	31.5	58.1
3	137.7	158.0	181.7	26.6	42.7	49.4
4	152.3	176.8	209.8	34.4	43.0	55.3
5	166.9	193.0	221.1	43.5	48.0	52.0
6	153.3	191.6	309.9	40.0	55.3	55.5
7	174.6	229.0	313.9	39.6	58.3	61.4
8	274.6	178.5	297.2	51.9	49.5	58.8
9	262.9	305.6	387.1	47.0	57.4	66.5
10	276.7	313.4	382.0	55.7	59.7	60.9
11	278.1	344.4	481.4	62.1	66.6	69.2
12	256.2	290.1	407.9	60.8	55.9	55.1

epididymidis is greatest between 2 and 3 months of age (Fig. 17). Increases in epithelial cell height in the corpus and caput epididymides are more gradual but there cell heights are similar to that in the cauda epididymidis by 6 and 10 months, respectively. These data show that the epithelial developmental changes commence in the cauda epididymidis and move up the epididymis towards

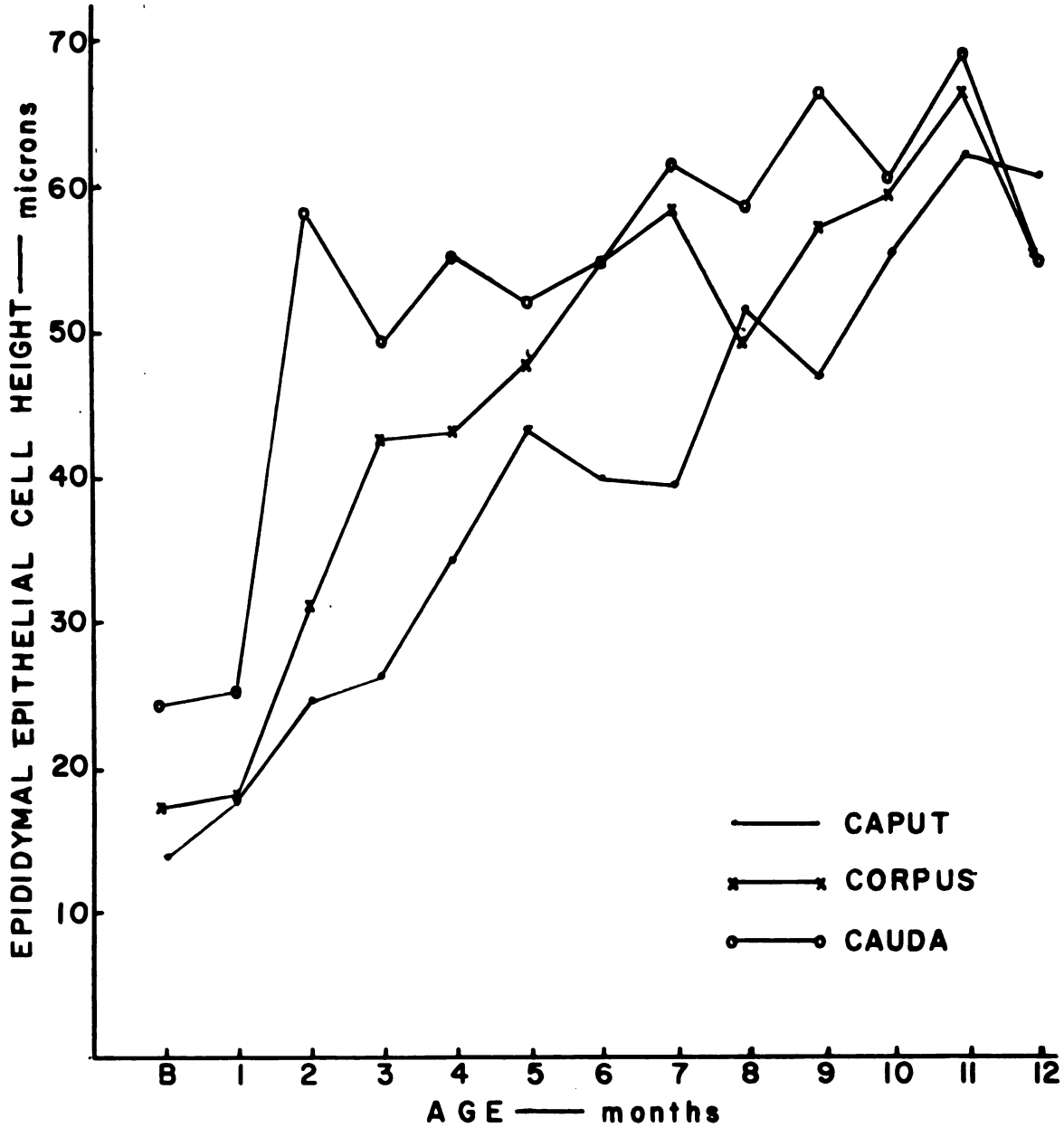

Figure 17.--Changes in the epithelial cell heights in the caput, corpus and cauda epididymides.

the testis. Since sperm move from the testis to the caput epididymidis, a descending manner of development was expected. However, the alternative conclusion from these data is the same as that reached by Abdel-Raouf (1960), although cell heights were greater in the Swedish bulls.

The epithelial cell height in the ductus deferens increases rapidly to 3 months of age, declines slightly to 5 months of age, and remains relatively constant thereafter (Table 18). Despite the fact that the ampulla develops a secretory role with advancing reproductive development these changes are not reflected in epithelial cell height. The month-to-month variation in this criterion is small and irregular. The development of the ampulla is best reflected by its weight which increases linearly from 2 to 8 months of age, increases dramatically between 8 and 9 months of age, and thereafter remains relatively unchanged to 12 months of age. The ductus deferens follows growth trends similar to those for the ampulla, but the rates of increase are smaller. In fact, the ductus deferens is heavier than the ampulla at birth but the situation is reversed after 3 months of age.

The changes with advancing age in the tubule diameter of the ductus deferens are irregular. Increases from birth to 1 month, 2 to 3 months, 5 to 6 months and 7 to 9 months of age are each followed by smaller decreases.

TABLE 18.--Changes in epithelial cell heights and weights of ductus deferens and ampulla and tubular diameter of the ductus deferens.

Age	Epithelial Cell Height		Organ Weight		Tubule Diameter
	Ductus Deferens	Ampulla	Ductus Deferens	Ampulla	Ductus Deferens
(months)	-----(microns)---		-------- g ------		(microns)
Birth	15.9	20.3	0.39	0.25	154
1	22.9	16.5	0.49	0.23	199
2	25.6	20.5	0.63	0.36	170
3	42.7	21.4	1.01	0.78	323
4	36.3	20.7	1.12	1.43	265
5	33.0	23.5	1.34	1.60	270
6	35.7	22.3	1.54	1.99	337
7	31.7	28.7	1.54	2.80	320
8	28.7	22.3	1.63	2.99	394
9	33.0	24.4	3.20	4.20	446
10	33.6	27.8	2.60	3.94	360
11	35.4	26.7	2.92	4.68	427
12	33.0	25.9	2.94	4.38	409

Consequently there is an increase in tubule diameter from birth to 12 months of age, but the pattern is irregular.

(e) Gonadal and Extra-Gonadal Sperm Numbers.--The estimates involving testicular homogenates include elongated spermatids (stages VI to VIII) as well as spermatozoa, as it was not possible to differentiate between them by procedures used in this study. These cell types were also included in the testicular counts made by Amann and Almquist (1961) in mature bulls.

Testicular sperm concentration increases dramatically between 7 and 8 months of age and continues to increase to 12 months of age (Table 19). The large value at 9 months partly reflects an unusually high concentration in one bull (62.17×10^6 sperm per g). The data for months 5 to 7 are indicative of the among animal variation in reproductive development during puberty. During this age-period sperm can only be detected in testicular homogenates from bulls showing advanced reproductive development.

To the author's knowledge, testicular sperm have not previously been reported in bulls less than 6 months of age. Hooker (1944) detected sperm histologically at 8.5 months of age, whereas Fossland (1954) did not detect sperm until 9 and 10 months of age in Jersey and Holstein bulls, respectively. However, Phillips and Andrews (1936) found sperm in the testis of a Herford bull at 7.5 months and Michatsch (1933) reported that solitary sperm appeared in the lumen of some seminiferous tubules at 6 months of age.

TABLE 19.--Gonadal sperm concentration and sperm numbers in bulls from 5 to 12 months of age.

Age	Number of Bulls	Sperm Concentration	Total Sperm
(months)		(10^6 sperm/g parenchyma)[a]	(10^9 sperm/ testis)[a]
5	1	4.24	0.15
6	2	6.80	0.34
7	3	4.26	0.27
8	5	27.87 ± 4.52[b]	2.62 ± 0.62
9	5	50.37 ± 3.80	7.80 ± 1.39
10	5	38.99 ± 1.77	5.41 ± 0.62
11	5	52.83 ± 4.19	8.59 ± 1.38
12	5	57.33 ± 5.97	10.69 ± 1.97

[a]Include spermatids (stages VI to VIII) and spermatozoa.

[b]± SE: only computed when average included 5 bulls.

In the present study, the "sperm" counts obtained from one 5 month old bull may have only comprised terminal stage spermatids. Nonetheless, this bull (No. 156) was well advanced in development because the weights of his anterior pituitary, seminal vesicles, testes, epididymides and ampullae were well above the respective averages for the five bulls of 5 months of age (Appendix I) and his seminal vesicular fructose content was also much higher

(Appendix III). The anterior pituitary of this animal was as heavy as the average gland weight in 9 month old bulls. This advanced development was not reflected in the bull's body weight. Although spermatogenesis was well advanced in the histological section of this bull's testis, sperm were not detected in any seminiferous tubules.

Almquist and Amann (1961) calculated that the average gonadal sperm concentration was 54.8 million sperm per gram in mature dairy bulls. This concentration is similar to that found in the 11 and 12 month old bulls in the present study. Thus, a major conclusion from this study is that Holstein bulls attain their mature rate of spermatogenesis by 11 months of age. After this point, increases in sperm production are due to increases in testicular weight and not to increased spermatogenesis per gram of testicular parenchyma. This conclusion would have significance in experiments wherein the objective is to increase sperm output in young bulls. One may most likely increase sperm output in young bulls by increasing testicular size, rather than the level of spermatogenesis.

In mature Holstein bulls, the gonadal sperm number 40.4 billion per testis (Almquist and Amann, 1961). Comparison of this value with the present data reveals that a 12 month old bull has a net testicular sperm production which is only about 25 percent of his ultimate mature

capacity. Attainment of this capacity is achieved by increasing testicular weight while maintaining a constant output per gram of testis.

At 6 months, only one (No. 150) of the five bulls had sperm in all segments of the excurrent ducts (Table 20 and Appendix IV), but from 8 months of age all the bulls had sperm in all the segments. The only 5 month old bull which had detectable gonadal sperm did not possess epididymal sperm. In mature sexually rested bulls, Almquist and Amann (1961) found that the caput epididymidis contained only about half as many sperm as the cauda epididymidis. However, in young bulls of 10 to 12 months of age this difference between the two segments is not as marked. This apparent change with advancing age would no doubt become more apparent as testicular sperm production increased and the cauda epididymidis assumed its role as the major storage organ for sperm awaiting ejaculation. Whereas the increase in testicular sperm from 12 months to maturity represents a four-fold increase the comparable changes in the caput, corpus and cauda epididymides represent five-fold, eight-fold and nine-fold increases respectively.

When Almquist and Amann (1961) attempted to deplete extragonadal sperm reserves and compared the epididymal counts to sexually rested bulls, only the cauds epididymides showed drastically reduced sperm numbers--13.7

billion sperm in depleted bulls and 37.6 billion in sexually rested bulls. Thus, even the depleted cauda epididymidis of a mature bull contains more than three times the number of sperm as the same epididymal segment of the yearling bull. Since the sex drive of the young bull is usually not as strong as that of mature bulls, fewer ejaculations are obtained in exhaustion studies with young bulls and, consequently, a proportional reduction similar to that found in mature bulls would not be expected. Therefore, the relatively low sperm output in young bulls reported by Bratton et al., (1959) and others is probably the result of low sperm reserves in the cauda epididymides.

The large variations in the sperm numbers of the deferent ducts and ampullae are reflected in their large standard errors (Table 20). In bulls of 8 months of age the ductus deferens contains more sperm than the ampulla, but the subsequent age increases are greater in the ampulla with the result that, from 10 months of age, the ampulla contains more sperm.

Data from Bratton et al., (1959) showed 12 month old bulls on a medium level of nutrition produced from 0.05 to 6.37 billion motile sperm per ejaculation. The average for the four bulls in that study was 1.95 billion motile sperm. Each bull was ejaculated once every 2 weeks. The percentage of motile sperm was not recorded in that experiment, but even with a normal rating (70 percent), it is

TABLE 20.--Extra-gonadal sperm numbers in bulls from 6 to 12 months of age.

Age	Epididymis				Ductus Deferens	Ampulla
	Caput	Corpus	Cauda	Total		
(months)	---------- ($X10^9$) ----------				--------- ($X10^6$) --------	
6	0.04(2)[a]	0.01(1)	0.02(1)	0.05	4.0(1)	6.0(1)
7	0.02(3)	0.01(2)	0.01(2)	0.03	4.9(1)	2.0(1)
8	0.21 ± 0.07[b]	0.09 ± 0.05	0.19 ± 0.01	0.49 ± 0.16	41.1 ± 14.4	26.0 ± 10.4
9	0.64 ± 0.24	0.08 ± 0.03	1.29 ± 0.57	2.00 ± 0.80	93.0 ± 53.8	83.8 ± 48.1
10	0.97 ± 0.23	0.27 ± 0.04	1.84 ± 0.41	3.07 ± 0.52	143.2 ± 43.4	154.5 ± 31.6
11	1.93 ± 0.69	0.23 ± 0.07	2.85 ± 0.85	5.01 ± 1.41	54.7 ± 9.2	213.8 ± 53.9
12	3.71 ± 0.55	0.57 ± 0.16	4.01 ± 0.69	8.29 ± 1.17	204.8 ± 52.9	970.6 ± 489.0

[a]Number of bulls, if less than 5.

[b]± SE: only computed if average includes 5 bulls.

apparent that if the Cornell Holsteins were similar to the present bulls, the sperm in the paired deferent ducts and paired ampullae could not account for the probable total sperm output. This conclusion shows that one ejaculation every 2 weeks will cause some epididymal depletion in yearling bulls. By contrast, the paired deferent ducts and paired ampullae in a sexually rested mature bull contain, on average, about 15.2 billion sperm (Almquist and Amann, 1961) and the total sperm in two ejaculates obtained after normal pre-ejaculation preparation and within 30 min of each other would not usually contain more than 15.2 billion sperm (Macmillan et al., 1966).

I stated in the Review of Literature that the termination of puberty would be defined by the detection of sperm in the cauda epididymides and deferent ducts. Applying this criterion to the bulls in the present study, puberty is terminated at 6 and 7 months of age in a few bulls which show advanced reproductive development (one out of five bulls in each age group) and in all "normally" developed bulls by 8 months of age. All five bulls in this age group had sperm throughout their excurrent ducts. Age changes in gonadal and extragonadal sperm numbers from 8 months are quantitative rather than qualitative.

D. CHANGES IN THE LUTEINIZING HORMONE-RELEASING ACTIVITY (LH-RF) OF THE HYPOTHALAMUS

The "Review of Literature" highlighted recent work which purported to show that the termination of puberty in the female rat was associated with dramatic changes in hypothalamic activity (Ramirez and Sawyer, 1966). The data obtained in this study with bulls are the first reported systematic attempt to determine whether another species exhibits similar changes.

The results (Table 21) show that the LH depletion activity exhibited by the hypothalami was not duplicated by the injection of a similarly treated cerebral cortical preparation at a dose level equivalent to twice that of the high dose levels of hypothalamic extract. That is, each rat was injected with a dose level of cerebral cortical extract equivalent in weight to one hypothalamus. This result means that the responses produced by the hypothalamic extracts are not due to neural proteins.

Another group of rats were injected with NIH-LH-B3 which had been inactivated by boiling for 10 min in 0.1 N hydrochloric acid and then removed by dialysis. If none of this LH had been inactivated and if all the LH had been found in the dialysate, each rat would have been injected with 20 ug LH. The rats which were injected with "inactivated LH" did exhibit a significant depression (4.85 percent) in ovarian ascorbic acid concentration ($p < 0.05$). Since an injection of 0.4 ug NIH-LH-B3 per rat reduced

TABLE 21.--The average concentrations of ascorbic acid in the ovaries of rats injected with one of two dose levels of hypothalamic extracts or with control preparations and their percentage deviation from saline injected controls.

Age	O. A. A. C.[a]		Deviation from Control	
	Low Dose[b]	High Dose[b]	Low Dose	High Dose
(months)	(ug/ml)	(ug/ml)	(%)	(%)
Saline Control	9.27 ± 0.14[c]		0	0
Birth	9.06 ± 0.42	9.29 ± 0.31	-2.27	+0.22
1	9.53 ± 0.40	9.54 ± 0.14	+2.80	+2.91
2	9.18 ± 0.19	9.37 ± 0.25	-0.97	+1.08
3	8.78 ± 0.45	9.06 ± 0.46	-5.29	-2.27
4	8.75 ± 0.34	9.29 ± 0.35	-5.61	+0.22
5	8.48 ± 0.21	7.98 ± 0.84	-8.52	-13.92
6	9.34 ± 0.45	8.65 ± 0.31	+0.76	-6.69
7	9.09 ± 0.33	8.53 ± 0.26	-1.94	-7.98
8	8.44 ± 0.25	7.65 ± 0.40	-8.95	-17.48
9	8.56 ± 0.62	6.94 ± 0.43	-7.66	-25.13
10	8.27 ± 0.25	7.84 ± 0.09	-10.79	-15.43
11	8.56 ± 0.37	6.95 ± 0.20	-7.66	-25.03
12	8.67 ± 0.25	7.39 ± 0.21	-6.47	-20.28
Cerebral Cortex	9.13 ± 0.15		-1.51	
Inactivated NIH-LH	8.82 ± 0.18		-4.85	
0.4 ug NIH-LH-B3	6.80 ± 0.32		-26.65	

[a] O. A. A. C. = Ovarian ascorbic acid concentration in homogenate containing 10 mg ovary per ml.

[b] Low dose = 0.25 hypothalamic equivalent per rat. High dose = 0.50 hypothalamic equivalent per rat.

[c] ± SE

concentration by 26.7 percent, the amount of LH which was not inactivated or removed represented less than 0.1 ug for every original 20 ug LH. This result means that 99.5 percent of the LH was destroyed or removed by boiling and dialysis. The levels of LH contamination in the hypothalami even before boiling and dialyzing would probably not have been very great, and, if the properties of native bovine LH are similar to the NIH preparation of bovine LH, the likelihood of LH contamination modifying the data for the hypothalamic extracts is negligible.

Therefore, the results obtained from rats injected either with a cerebral cortical extract or LH subjected to boiling in acid and dialysation show that the responses derived from the hypothalamic preparations were due to LH-RF and were not the result of non-specific reactions or LH contamination.

That there are age changes in LH-RF content is apparent from the graphical representation of the data (Fig. 18). Neither dose level of the hypothalamic extracts prepared from the bulls aged from birth to 4 months produced a response which was significantly different from the rats injected with saline. Furthermore, the responses for these age groups are not valid because the low dose levels produced a greater depression in ascorbic acid concentration than the high dose levels. From 5 to 12 months of age, the responses are valid and the curves for the two dose levels are rather similar.

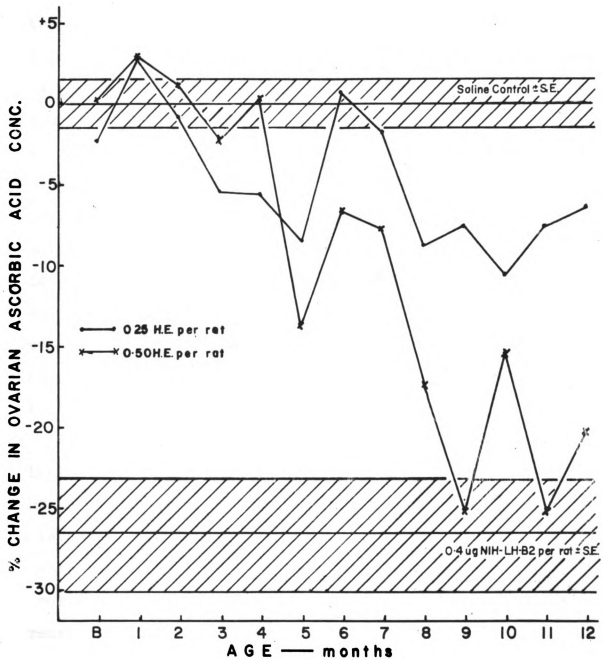

Figure 18.--Changes in the ovarian ascorbic acid concentration of pseudo-pregnant rats produced by two dose levels of hypothalamic extracts derived from Holstein bulls to 12 months of age.

Although significant levels of LH-RF were not detected in the hypothalami from bulls less than 5 months of age, this does not necessarily mean that this releasing factor was not present as the assay is of unknown sensitivity. In the intact animal, LH-RF is released into the portal vessels of the median eminence and is then transported in the blood to the pituitary. Dilution of the releasing factor would be minimal in this short distance. However, in the assay animal the releasing factor, which may have been altered or partly inactivated by the preparative procedures, is injected into the femoral vein and would be considerably diluted before it reached its target organ, the anterior pituitary. Thus, the assay used is undoubtedly very insensitive relative to a similar quantity of LH-RF released from the hypothalamus in its normal anatomical location.

Five months was the youngest age at which the levels of LH-RF in the hypothalami from Holstein bulls were sufficient to produce a significant response in the Parlow rat. The levels of LH-RF are lower at 6 and 7 months of age and then increase markedly to 9 months of age. However, the results from low dose levels only show a comparable increase to 8 months of age and do not change greatly thereafter. The response obtained with the hypothalami from 10 month old bulls are inconsistent because the low dose level resulted in a response slightly greater than

the responses produced by hypothalami from bulls of 8, 9, 11 and 12 months of age, whereas the response to the high dose level was significantly less than those produced by hypothalami from bulls of 9 and 11 months of age ($p < 0.05$). The fluctuations of LH-RF levels in mature intact males have not been determined, but since the male does not exhibit cyclic reproductive activity like the female, fluctuations in LH-RF activity in the post-public male would not be anticipated.

The general pattern of these results reveals undetectable levels of LH-RF through 4 months of age, a sudden increase at 5 months of age, a decline at 6 months, an increase to 8 months and little change thereafter to 12 months of age. Since no studies have been made on LH-RF activity in hypothalami from mature Holstein bulls, it cannot be concluded from this study that 8 to 12 month-old bulls have attained their maximum LH-RF levels.

The interpretation of these results in light of the reports in the literature is rather complicated. Most of these reports were based on results obtained with rats and extrapolation to the bull may not be justified. Similarly, conclusions based upon female rats may not be applicable to male rats. Halász and Pupp (1965) severed all nervous pathways to the hypophystropic area of the median eminence in rats and showed that whereas testicular function was maintained, the ovulatory ability

was terminated, because the ovaries remained in their pre-operational state. Gorski (1966) in his comprehensive review, interpreted this data by Halász and Pupp (1965), together with his own data involving lesioning and androgen sterilization, to show that in intact male rats and androgen sterilized female rats, testosterone had eliminated the cyclic LH releasing properties of the preoptic area during the first post-natal week. Thus he inferred that the role of hypothalamic LH-RF in the male is to maintain a tonic release of LH which in turn maintains testicular function at a relatively constant level.

Gorski (1966) considered that loss of this regulatory capacity was possibly because the ovarian hormones regulate the preoptic area as well as the ventralomedial nuclei which maintain tonic gonadotropic release. However, Gorski's conclusions may be an oversimplification of the sexuality in the hypothalamus. Takeisnik et al., (1966) showed that copulation in rats produced a dramatic rise in the plasma LH in both sexes within 10 min. Comparable changes in plasma FSH were not detected but FSH potency increased significantly 4 hr after copulation in the males.

Piacsek and Meites (1966) and Mittler and Meites (1966) castrated mature male rats and measured the levels of LH-RF and FSH-RF, respectively, in the hypothalamus with and without replacement steroidal therapy. In both cases, castration increased the concentration of hormone in the pituitary and the level of releasing activity in the hypothalamus. Injections of testosterone propionate depressed

pituitary hormone potencies and hypothalamic releasing activities. The conclusions reached was similar in both studies--testosterone propionate injected into castrate rats appears to reduce the levels of gonadotropic releasing factors in the hypothalamus and these reductions are associated with a reduction in hormonal potency in the pituitary. Although endogenous testosterone may exert an effect similar to exogenous testosterone propionate, steroids are only one factor which influence hypothalamic releasing activity. Corben and Cohen (1966) showed that hypothalamic implants of LH reduced pituitary LH levels and suggested that the mode of action was by reducing LH-RF levels. Ramirez and Sawyer (1956a, and 1966) and Chowers and McCann (1965) also advanced the hypothesis that LH-RF secretion is controlled by the internal negative feedback influence of circulating LH.

In addition to the possible influences of the gonadal steroids and the circulating levels of gonadotropins, the LH-RF activity of the hypothalamus may also increase with age. Although Ramirez and McCann (1963) could not detect differences in LH-RF activity of hypothalami removed from mature and immature rats, Ramirez and Sawyer (1966) subsequently showed that in female rats the LH-RF levels increased steadily from 28 to 36 day of age and then showed a dramatic peak which was associated with vaginal opening. Injections of estradiol benzoate reduced the age at which the peak occurred to 33 days of age.

The ability of steroids other than estradiol benzoate and testosterone propionate to alter the levels of hypothalamic LH-RF have not been reported. Although Gorski (1966) showed that androstenedione could not produce the androgen-sterility syndrome, this steroid may still influence the hypothalamus. This hypothesis is attractive in view of the data of Ryan and Philpott (1967) who showed that injections of androstenedione could dramatically reduce the elevated post-castrational levels of LH in the rat pituitary, although the effects of comparable injections of testosterone were several times greater.

When comparing hypothalamic levels of LH-RF and pituitary levels of LH, the possibility that the hypothalamus stimulates the synthesis of pituitary LH as well as causing increases in its rate of release must be considered. This dual role of the hypothalamus has been shown with GH (Meites, 1964). Kuroshima et al., (1966) found that injection of beef stalk-median eminence extract at 0.1 equivalents per rat caused a 45 percent reduction in pituitary FSH within 30 min, but preoperational levels of FSH had been restored by 60 min suggesting that FSH synthesis occurred together with or soon after FSH release.

Because of these numerous modifying influences, the fluctuations in the LH-RF activity of the hypothalmi obtained from bulls may not be directly reflected in pituitary and plasma levels of LH. However, the following conclusions are justified:

(i) The large fluctuations in the potency and content of LH in the pituitary prior to 5 months of age are not associated with dramatic changes in hypothalamic levels of LH-RF.

(ii) The increase in LH-RF from 6 to 8 or 9 months of age is associated with a marked decline in the LH potency and content of the pituitary and a rapid increase in plasma LH.

(iii) From 8 to 12 months of age the pituitary LH potency, plasma concentration and the levels of LH-RF show only minor changes, but total pituitary LH and total plasma LH do not stabilize until 9 and 10 months of age, respectively.

When discussing the results relating to total plasma LH, it was suggested that the hormonal changes associated with reproductive development may comprise two distinct phases of change, with the first lasting from 2 to 4 months of age and the second from 6 to 9 months of age. Between 4 and 6 months of age there is a plateau. The peak in the LH-RF at 5 months of age and the decline to 6 months of age may well be part of this biphasic phenomenon.

Although comparisons of results obtained in different laboratories must be made with caution, it is of interest to note that Ramirez and McCann (1963) found that a dose level equivalent to 10 mature rat stalk-median eminences per assay rat produced a 20 percent decline in the ovarian ascorbic acid concentration. A similar response was

produced by 0.5 bull (9 to 12 months of age) hypothalamic equivalents per rat. This indicates that the level of LH-RF per hypothalamus in bulls in this age bracket is about 20 times greater than in mature rats.

E. CHANGES IN THE ACCESSORY REPRODUCTIVE ORGANS, THYROID, ADRENAL AND THYMUS

(a) Seminal Vesicles.--The development of the seminal vesicles has been extensively studied in the bull mainly because their development and their synthesis and secretion of fructose and citric acid were presumed to indicate changes in androgen levels. This hypothesis was substantiated by Mann et al., (1949) who showed that in castrate bulls, the output of these two seminal vesicular products was dependent upon the dose level of exogenous testosterone. They also suggested that fluctuations in citric acid or fructose outputs by an intect bull were due to periodic changes in androgen titers.

The changes in weight, total DNA and total RNA (Table 22) are illustrated together to demonstrate the similarities in these responses (Fig. 19). The declines from birth to 1 month are no doubt due to the removal of the residual influence of the placental hormones on the neonatal calf (Nalbandov, 1958). The loss in each of these growth parameters is regained by 2 months of age. Seminal vesicular DNA content increases dramatically

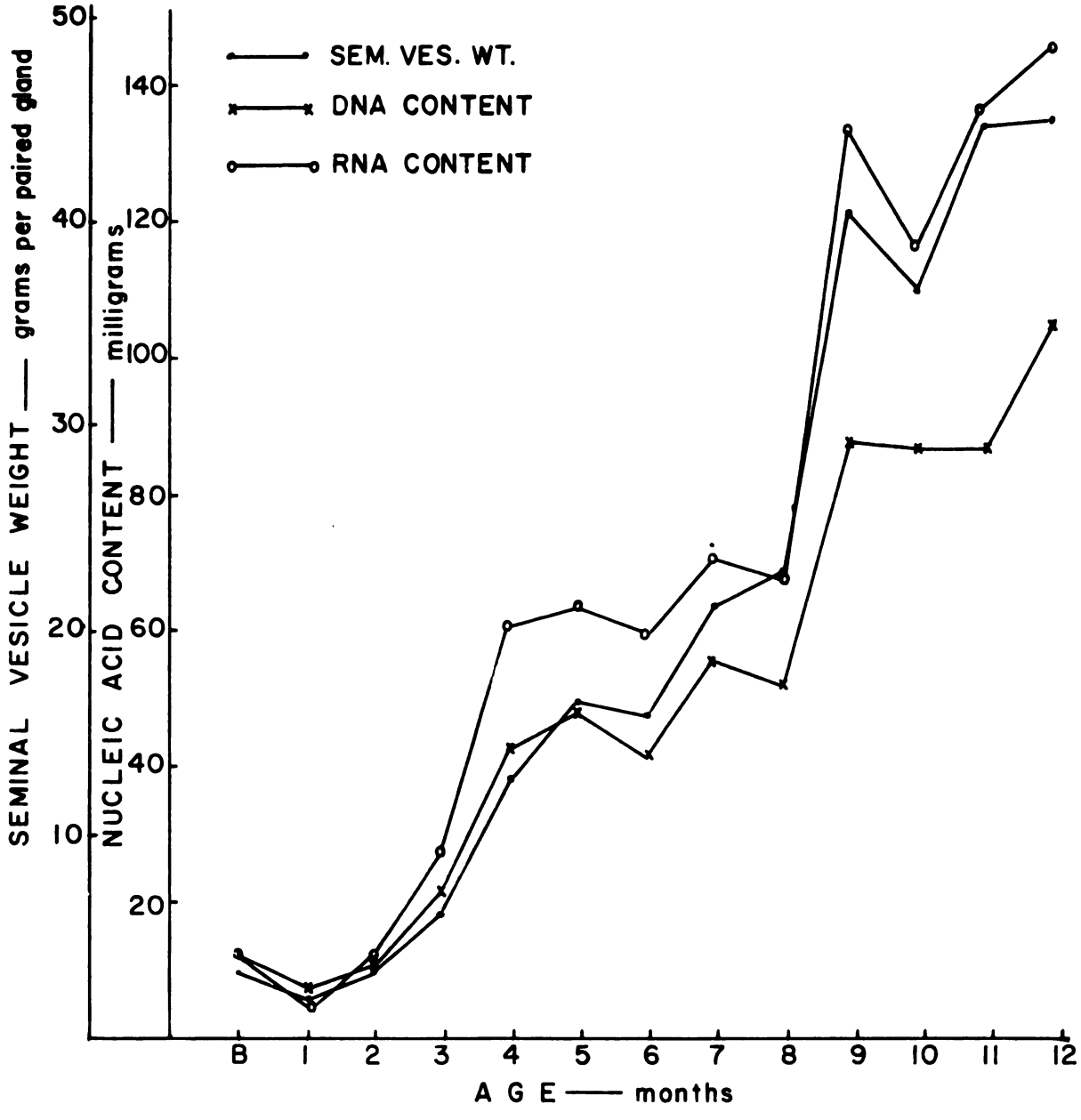

Figure 19.--Changes in the weight of the paired seminal vesicles and their DNA and RNA contents.

TABLE 22.--Changes in the weight of the paired seminal vesicles and their DNA, RNA, citric acid and fructose contents.

	Weight	Total DNA	Total RNA	Total Citric Acid	Total Fructose
(months)	(g)	----------	----------	(mg)	----------
Birth	3.29	11.93	12.37	2.17	0.26
1	1.95	5.45	4.38	1.43	0.12
2	3.08	10.77	11.43	2.86	0.51
3	5.95	21.29	27.85	5.09	1.61
4	12.56	42.75	60.09	11.11	4.82
5	16.24	45.79	62.74	16.56	8.89
6	15.61	40.58	58.68	17.07	12.80
7	21.12	55.68	69.91	46.26	20.74
8	22.71	50.79	67.25	54.79	31.82
9	40.18	87.49	132.37	76.11	101.90
10	36.20	86.65	114.19	81.81	59.52
11	44.15	86.08	135.27	108.83	98.52
12	44.61	103.90	148.90	130.88	81.50

between 2 and 4 months, does not change from 4 to 6 months, increases again to 9 months of age and thereafter does not change very much. The RNA curve is very similar to that for DNA, but the overall increase is greater than that for DNA.

Abdel-Raouf (1960) found a linear increase in seminal vesicular weight in Swedish Red-and-White bulls between birth and 16 months of age. However, his results show two periods during which weight changes were small. The periods were from 24 to 32 weeks (5.5 to 7.5 months) and from 44 to 56 weeks (10 to 13 months) and both are at similar ages as the plateaued periods of seminal vesicular growth in the present bulls.

Rabinovitch and Lutwak-Mann (1951) showed that injections of testosterone produced an initial increase in seminal vesicular RNA and a subsequent increase in DNA in castrate rats. Because of this preferential response, the data from the bulls was examined to see whether the changes in RNA preceded those for DNA. The accompanying diagram (Fig. 20) shows that the RNA/DNA ratio increases from 1 to 4 months of age but thereafter does not change. This means that the increases in the RNA content are proportionately greater than DNA changes to 4 months of age, but from 4 months of age changes in androgen levels produce proportionally similar changes in gland growth (DNA) and protein synthesizing potential (RNA).

The changes in seminal vesicular fructose and citric acid contents reflect neither the changes in RNA nor DNA (Fig. 21). The increases in fructose and citric acid are gradual and not very great from 2 to 6 months, but both components increase rapidly from 6 to 7 months of age.

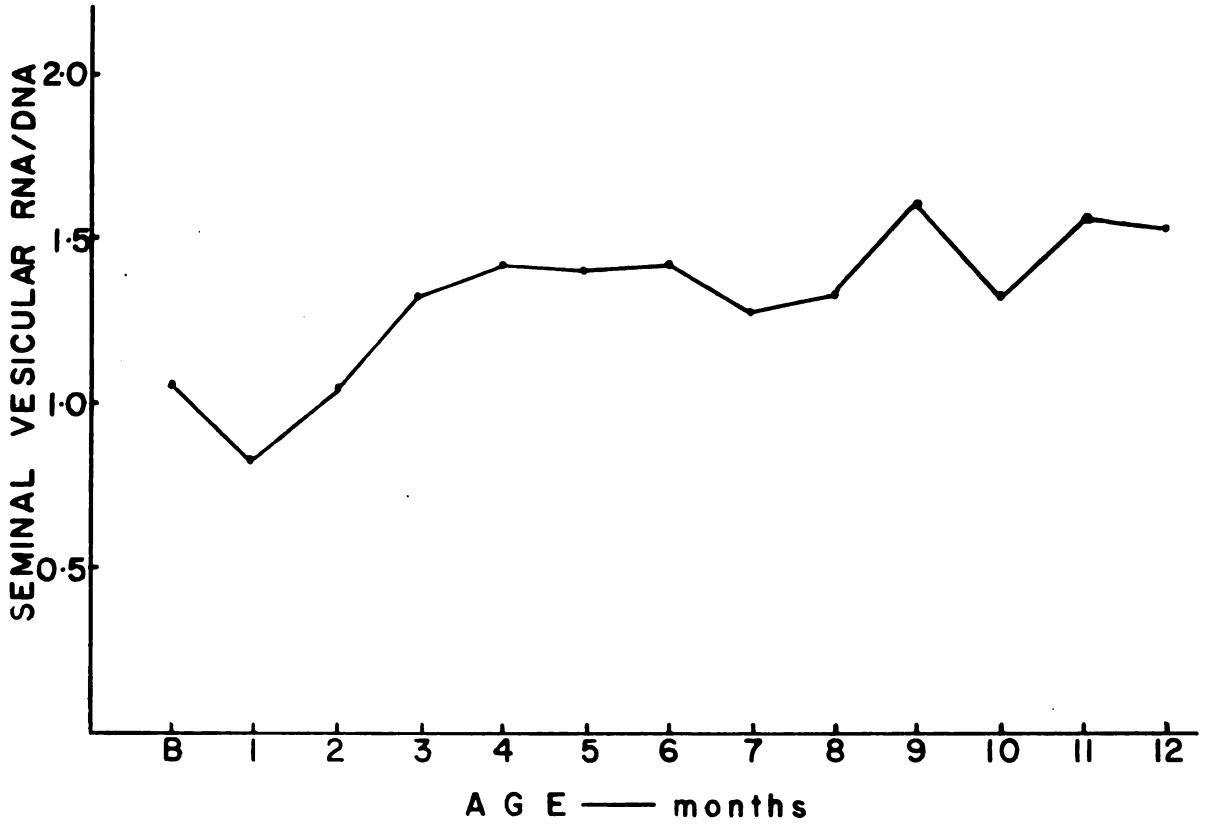

Figure 20.--Changes in the seminal vesicular RNA : DNA ratio.

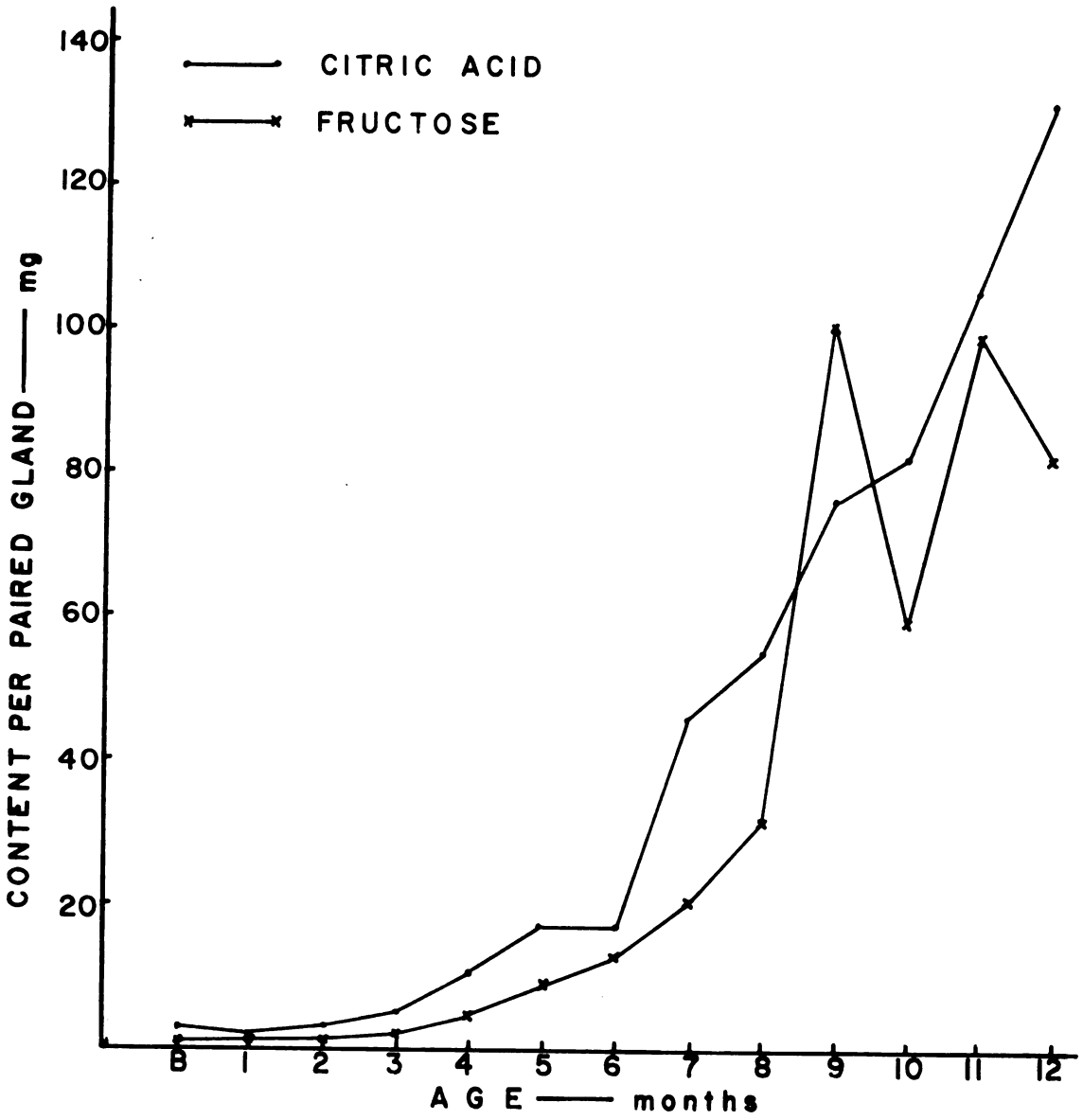

Figure 21.--Changes in the citric acid and fructose contents of the paired seminal vesicles.

The increase in citric acid content during this latter period is greater than the overall increase during the first 6 months. Abdel-Raouf (1960) noted similar changes at similar ages in Swedish bulls. In the present bulls, as in the Swedish bulls, the greatest increase in fructose content occurs between 8 and 9 months of age. In both breeds, the fructose content is greater at 9 months than at any age to 12 months. One major difference between the breeds is that in Swedish bulls the fructose content is considerably greater than the citric acid content in bulls which are more than 8 weeks old, whereas in the present Holsteins the citric acid content is greater except at 9 months.

The increase in seminal vesicular fructose and citric acid from 6 to 7 months of age may be due not only to changes in androgen titers, but also to the onset of seminal vesicular secretory activity. Abdel-Raouf (1960) could not extrude any liquid from the seminal vesicles from bulls aged less than 6 months of age. A similar procedure was not performed with the present bulls because the process could not be satisfactorily standardized. However, the previously mentioned similarities between the Swedish and present bulls suggest that Abdel-Raouf's observations should be applicable to the Holsteins. Of note is the fact that Abdel-Raouf (1960) found that, whereas the fructose contents of the glands were greater,

the secretions had greater concentrations of citric acid. He suggested that the glandular epithelium has a greater capacity for storing fructose and for secreting citric acid.

The changes in seminal vesicular activity are not reflected by changes in the cell height of the secretory epithelium (Table 23). Dramatic changes were not expected because Abdel-Raouf (1960) had made similar observations and a similar conclusion.

The results obtained for seminal vesicular DNA and RNA suggest that both the growth and the protein synthesizing potential show two distinct phases of increase. The first phase is from 2 to 4 months and the second from 8 to 9 months of age. Changes between 4 and 8 months, and after 9 months of age were not so great. These results are further evidence that the reproductive development of the Holstein bull has two phases of accelerated growth with a plateau between them.

(b) Penile Length.--The penis of each bull was measured from the tip of the glans penis to the point of attachment to the pelvis. The sigmoid flexure, when it was present, was extended. The results of these measurements (Table 24) reveal that the increase in length is generally linear to 9 months of age. The changes in length after 9 months are small. These results lead to the conclusion that the Holstein bull's penis attains its mature length by 9 months

TABLE 23.--Changes in the cell height of the secretory epithelium of the seminal vesicles.

	Age (months)												
	Birth	1	2	3	4	5	6	7	8	9	10	11	12
Cell Height (microns)	23.6	15.3	22.3	23.5	23.5	22.6	25.1	28.7	24.7	30.5	25.4	26.6	35.6

TABLE 24.--Increase in the length of the penis from birth to 12 months of age.

	Age (months)												
	Birth	1	2	3	4	5	6	7	8	9	10	11	12
Length (cm)	30.7	31.7	37.9	42.9	45.5	64.8	55.9	62.5	69.1	80.0	70.6	82.6	81.8

of age. In Swedish bulls this age is 8 months (Abdel-Raouf, 1960).

(c) The Thyroid Gland.--The two measured thyroidal parameters are thyroid weight and the acini epithelial cell height. The results obtained (Table 25, and Fig. 22) reveal that age changes in thyroid weight are irregular and the standard errors erratic (Appendix VI). The latter fact reflects the large differences in the weights of the thyroids obtained from bulls of the same age. The graph suggests a negative correlation between average weight and cell height. However, when all 65 glands are considered, the correlation coefficient between weight and cell height is only -0.26, a value which is significant ($p < 0.05$), but not of great predictive value.

However, there are obvious cytological changes between birth and 12 months of age. Photomicrographs (Fig. 23) show that in young bulls, the acini are large and filled with colloidal secretion, but with increasing age, the acini become smaller and by 12 months of age are very small and circular.

Comparison of the data in the present study with that obtained by Desjardins (1966) from Holstein heifers shows that sex does not appear to influence average thyroidal weight but the acini cell height is much less in heifers from 1 to 5 months than in bulls of comparable

TABLE 25.--Changes in the weight of the thyroid and acini epithelial cell height in Holstein bulls and heifers.

	Thyroid Weight		Epithelial Cell Height	
	Bulls	Heifers	Bulls	Heifers
(months)	g		microns	
Birth	10.9	8.6	9.0	12.4
1	21.6	15.6	9.1	5.4
2	15.3	9.7	8.1	5.7
3	26.4	12.0	7.8	4.8
4	16.8	25.0	8.6	6.9
5	12.6	16.5	9.7	8.4
6	14.0	13.3	9.2	11.3
7	10.1	12.7	9.9	9.9
8	20.6	13.3	8.6	9.2
9	13.8	15.3	9.9	10.0
10	13.6	16.6	10.6	10.6
11	15.7	15.0	11.6	13.0
12	15.7	19.1	9.7	12.1

age. It is of interest to note that in heifers of 4 months of age, the thyroid was much heavier than at any other age to 12 months. A comparable peak occurs in bulls of 3 months of age. Since Desjardin's heifers received management which was similar to that for the present bulls, the similar results for thyroid weight in the young animals of both sexes, supports the explanation

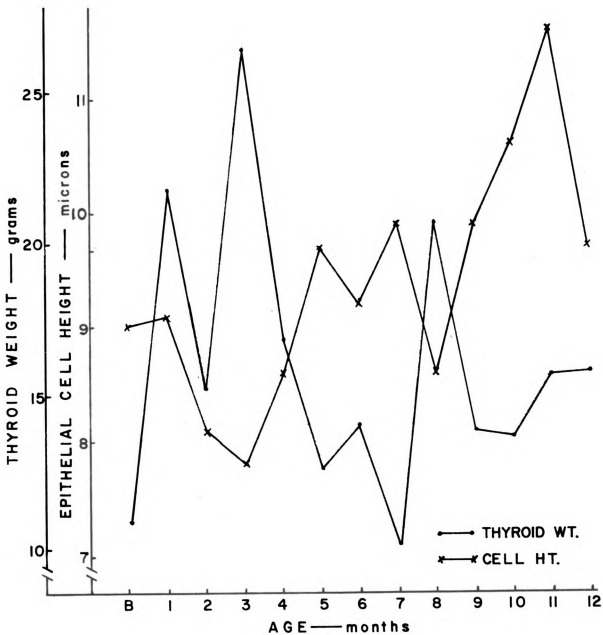

Figure 22.--Changes in thyroid weight and acini epithelial cell height.

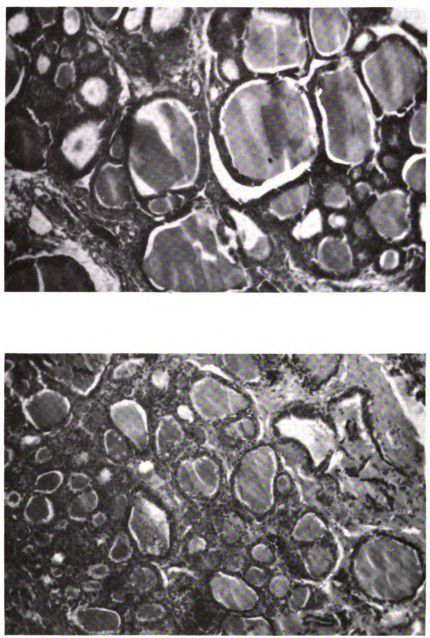

Figure 23.--Photomicrographs showing differences in thyroidal acini size in Holstein bulls at 4 months (a) and 10 months (b) of age (x150).

advanced by Desjardins (1966). He suggested that the young calves had received iodine definient diets. However, the acini cell height in heifers showed a dramatic decline which was not duplicated in the results obtained from the bulls.

The changes in the two thyroidal parameters did not appear to be associated with changes in body weight or with reproductive development.

(d) Adrenal Glands.--The weight of the paired adrenal glands increases linearly from birth to 10 months of age with only slight weight changes thereafter (Table 26). By contrast, the adrenals of the Swedish Red-and-White bulls follow a quadratic growth curve (Abdel-Raouf, 1960). Consequently, the adrenal glands of young Swedish bulls are heavier, but from 9 months of age the weights are similar to Holstein bulls of comparable age.

A comparison with the adrenal weight data obtained in heifers by Desjardins (1966) shows similar age changes. In fact, the monthly averages for bulls and heifers are mostly within a gram. However, the width of the zona glomerulosa is consistently greater in bulls than in heifers from 2 to 11 months of age. Although the combined width of the zonas fasiculata and reticularis is greater at most ages in bulls than in heifers, the proportional differences are not as great as the differences between

TABLE 26.--Changes in the weight of paired adrenal glands and the widths of the zona glomerulosa and the zonas reticularis--fasiculata in Holstein bulls and heifers.[a]

Age	Weight		Glomerulosa Width		Ret.-Fas. Width[b]	
	Bulls	Heifers	Bulls	Heifers	Bulls	Heifers
(months)	------- g ------		---------- microns ---------			
Birth	2.94	3.5	172	225	803	866
1	3.32	3.2	157	188	844	754
2	4.42	4.3	194	181	870	1052
3	5.71	4.8	208	181	941	994
4	6.86	7.2	215	174	1109	1012
5	8.77	7.7	188	147	848	1073
6	8.45	7.5	185	147	1140	1039
7	8.95	9.1	229	153	1183	967
8	9.87	9.4	204	171	1052	1043
9	11.92	9.8	210	177	1245	1049
10	13.12	12.0	204	179	1163	1142
11	13.08	12.9	227	189	1200	1198
12	13.63	14.5	206	222	1218	1217

[a]Heifer data derived from Desjardins (1966).

[b]Combined width of zonas reticularis and fasiculata.

the sexes in the width of the zona glomerulosa. However, the increases in the measured widths are more erratic in bulls than in heifers. Desjardins (1966) suggested that

the combined width of the zonas fasiculata and reticularis increased with the commencement of detectable estrous cycles. In bulls none of the changes in the adrenal parameters appear to be associated with reproductive development.

(e) Thymus Gland.--The weight of the thymus gland of bulls (Table 27 and Fig. 24) increases dramatically from 1 to 4 months of age and then shows a decline to 7 months of age. Between 7 and 12 months there is a slight weight increase with age. These results are in dramatic contrast to those obtained by Desjardins (1966) in heifers (Table 27 and Fig. 24). He found that thymus weight increased steadily from 1 to 12 months of age and concluded that reproductive development in heifers was not related to changes in thymus weight. His results were in contrast to those obtained with female laboratory animals in which thymus weight declines with advancing age (Defendi and Metcalf, 1964).

Martin (1964) reported that sham-operational thymectomy in male rats of 6 weeks of age resulted in a significant depression in ventral prostrate weights by 3 weeks. However, ventral prostrate weights in thymectomized and unoperated rats did not differ. These results suggest that an interaction between the thymus gland and post-operational stress produced the decline in contrast to any direct influence by the thymus on reproductive

TABLE 27.--Changes in the weight of the thymus gland in Holstein bulls and heifers to 12 months of age.

	Age (months)												
	Birth	1	2	3	4	5	6	7	8	9	10	11	12
Bulls	61	78	251	335	429	279	239	144	162	181	180	235	175
Heifers[a]	101	69	152	292	233	341	377	350	326	367	553	393	591

[a]Data derived from Desjardins (1966).

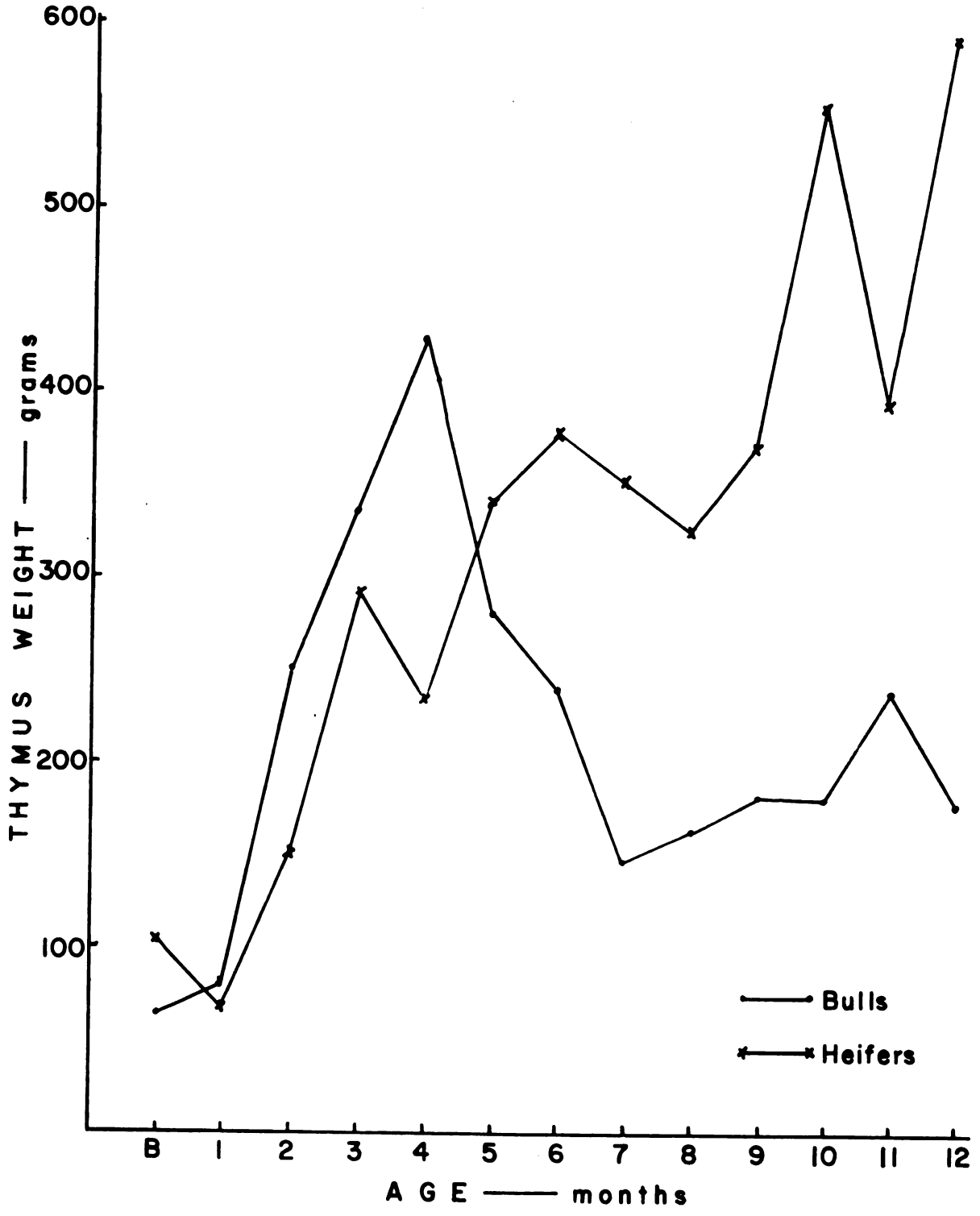

Figure 24.--Changes in the weight of the thymus gland in Holstein bulls and heifers to 12 months of age.

development. Although the decline of thymus weight in the female rat has been hypothesized to be due to increasing levels of estrogen, the present results obtained with bulls may not be the exclusive result of increasing androgen. On the contrary, seminal vesicular development and increases in total plasma LH suggest that puberty has commenced by 3 months of age, and if testicular androgens were responsible, increasing levels of steroids would be expected to continue to depress thymus weight at all ages beyond 3 months. But thymus weight increases in bulls from 7 to 12 months of age.

Hegyeli et al., (1963) found that extracts of prepuberal calf thymus could sterilize adult female mice and suggested that the thymus gland may exert a restrictive effect on the debut of puberty. If this theory was applicable to bulls, one would expect to find significant negative correlations between thymus weight and parameters of sexual development such as testicular and seminal vesicular weights and the fructose content of the seminal vesicles in bulls between 2 and 6 months of age, the age period when thymus weight shows its greatest changes. Calculations show that none of the correlation coefficients between these parameters and thymus weight is significant even after corrections for age differences (from $r = -0.25$ to $r = 0.26$).

The conclusions from the present data and the above calculations are that the age changes in the weight of the thymus gland in the Holstein bull do not appear to be

associated with reproductive development. However a comparison of Figs. 11 and 24 show that the age trends in the pituitary levels of GH are very similar to the age-weight changes in the thymus. The possibility of a relationship between GH and thymus weight in dairy cattle has not been reported in the literature.

F. GENERAL DISCUSSION

Comparison of the body weight data with figures quoted by Morrison (1956) indicates that in terms of this parameter, the Holstein bulls used in this study constituted a typical sample. Consequently, the remaining data obtained from these bulls are probably applicable to Holstein bulls in general and should be useful as a reference source for subsequent research involving young bulls.

Since both LH and FSH are necessary for the maintenance of normal testicular function, one might expect a significant correlation in the pituitary potencies of the two gonadotropins. The synergistic action of the two hormones was demonstrated by Greep et al., (1936) who found that the increase in testicular weight in intact or hypophysectomized immature rats produced by FSH was potentiated by the addition of LH. However, the correlation coefficient for the monthly averages of FSH and LH potencies of the pituitaries from the Holstein bulls in the present experiment was not significant ($r = 0.39$). The data obtained

from the five day-old bulls was not included in these calculations because residual maternal hormones may have influenced potency estimates. Nonetheless, there are noteworthy similarities in the age trends in these two hormones. The maximum potencies of pituitary LH and FSH occur at 1 month (Fig. 4) and 2 months of age (Fig. 8), respectively. Pituitary content of both hormones is greatest at 5 to 6 months of age. Whereas potency estimates for both hormones show gradual declines to 12 months of age, the changes in pituitary content are not as marked. It is of interest to note that Desjardins (1966) reported that the pituitary FSH potency is greatest in Holstein heifers at 1 month of age.

Similar trends with age in FSH potencies have also been reported in female rats by Hoogstra and Paesi (1955) and Kragt and Ganong (1967). Hoolandbeck et al., (1956) reported that total pituitary gonadotropic potency in gilts also declined with age. They speculated that the greater potencies in the younger animals were due to relatively greater levels of FSH than LH, and that the initiation of cyclic activity at puberty was the consequence of increased LH secretion which yielded a more functionally balanced FSH-LH ratio. A similar interaction between the two gonadotropins may also occur in female rats. The pituitary potency of FSH declines from 77.1 ug per mg at 20 days of age to 7.0 ug per mg at 35 days of age, (Kragt and

Ganong, 1967), whereas the LH potency gradually increases during this age period but shows a precipitious decline just prior to vaginal opening (Ramirez and Sawyer, 1965a).

If one may assume that the pituitary level of FSH in Holstein bulls is proportionally related to the blood plasma level of FSH, then the blood plasma level of FSH would increase to 5 or 6 months of age which is the age at which the blood plasma level of LH begins to show a dramatic increase (Fig. 6). The possibility that levels of plasma FSH are high from 2 to 6 months of age is supported by the testicular data. Testicular weight and nucleic acid contents begin to increase at increasing rates from 3 months of age. These increases may be due to FSH, because Simpson et al., (1951) showed that in the rat the growth of the testis is predominately due to FSH, and the increase in testicular volume produced by FSH is a reflection of an increase in the size of the seminiferous tubules. In Holstein bulls the diameter of the seminiferous tubules increases from 1 month of age (Fig. 15).

The greater increase in testicular weight in Holstein bulls from 6 to 9 months of age is probably the result of the increasing levels of plasma LH which synergize with the FSH (Greep et al., 1936). Nelson (1952) considered that apart from growth in the tubular diameter in the rat testis, FSH may only be responsible for the proliferation of spermatogonia and primary spermatocytes. That is, LH and FSH are both necessary for spermatogenesis. A similar

explanation may be applicable to testicular development in the Holstein bull as the increase in blood plasma LH at 6 months occurs at the same age at which sperm or terminal stage spermatids are detected in some testicular homogenates (Table 19). The increases in the amount of plasma LH to 9 months of age is associated with increased gonadal sperm production as revealed by sperm per gram of testicular parenchyma.

The relationship between hypothalamic levels of LH-RF and plasma levels of LH suggests that the increase in the latter parameter from 6 to 10 months of age is associated with an increase in LH-RF activity (Fig. 19). However, the increase in the level of LH-RF between 4 and 5 months of age and the decline between 5 and 6 months of age are not reflected in plasma LH. Prior to 5 months of age, significant levels of LH-RF could not be detected in the hypothalamus of the Holstein bull. However, some LH is apparently being released by the anterior pituitary as the amount of plasma LH increases from 2 to 4 months of age and then is maintained at this level of release to 6 months of age. These changes in plasma LH apparently influence testicular function which, in turn, is reflected by changes in seminal vesicular growth. The weight of the paired seminal vesicles also increases from 2 to 4 months of age (Fig. 19). These trends are also reflected in RNA and DNA values, with the increase in RNA content from 4 to

6 months being greater than the increase in DNA content (Fig. 20).

Lindner and Mann (1960) found that the concentration of testosterone in testes obtained from bulls older than 10 months of age varied from 19 to 437 ug per 100 g. In younger bulls of prepuberal age, the variations were at least as great, and in individual calves from 3.5 to 5.5 months of age no correlation could be established between the testicular androgen content and either seminal vesicle weight or secretory activity. However, their results clearly showed that in bulls less than 4 months of age, the testicular androstenedione (androst-4-ene-3,17-dione) content was 10 times greater than the testosterone content. From 6 months of age this relationship was reversed. In the present study, this latter age coincides with an increase in the amount of plasma LH which would stimulate testosterone synthesis.

The youngest age at which testosterone was detected in bull testes was 2 months (Lindner and Mann, 1960). This is also the age at which seminal vesicular growth commenced in the Holstein bulls used in the present study. Lindner and Mann infer that the greater levels of androstenedione in the testes of prepuberal bulls are probably ineffective in stimulating either seminal vesicular growth or secretory activity, but merely serve as a precursor of testosterone or even estrogen. Mann et al., (1949 and 1960) have

demonstrated that fructose and citric acid levels in semen and seminal vesicles are influenced by testosterone, and Lindner and Mann (1960) calculated that in bulls older than 9 months of age the correlations between the log of testicular testosterone and seminal vesicular weight and secretory activity were highly significant ($r = 0.59$ to 0.89). Nonetheless, androstenedione may still have an effect on seminal vesicular growth as Ryan and Philpott (1967) produced significant increases in seminal vesicular weight in castrated male rats with exogenous androstenedione. Thus, the growth of the seminal vesicles observed in the Holstein bulls between 2 and 4 months of age in the present study may have been due to androstenedione. The subsequent accelerated seminal vesicular growth from 6 to 9 months of age and dramatic increases in the levels of citric acid and fructose from 6 to 7 months of age (Fig. 21) are no doubt due to the increased levels of plasma testosterone.

The data for seminal vesicular growth, plasma LH, and possibly LH-RF suggest that the reproductive development of the Holstein bull to 12 months of age has two accelerated growth phases with an intervening period of less rapid growth. The first rapid growth phase occurs between 2 and 4 months of age and the second between 6 and 9 months of age. The changes during the latter phase are greater and appear to be associated with increased

hypothalamic activity which in turn is reflected by increased levels of plasma LH. The increased release of LH from the pituitary presumably stimulates increased testosterone synthesis and this produces increased seminal vesicular growth and promotes secretory activity in the seminal vesicles. The increased levels of plasma LH also promote spermatogenesis and, by 11 months of age the rate of sperm production (sperm per gram of testis) is similar to that found in mature Holstein bulls (Table 19).

Thus, puberty in the Holstein bull is that period between 2 and 9 months of age. During this period many reproductive parameters show their greatest rates of increase. They include testicular weight, width, length, shape and nucleic acid content, seminiferous tubule diameter, seminal vesicular weight, nucleic acid content, and fructose and citric acid contents, ampulla weight, vas deferens weight, hypothalamic LH-RF activity and plasma levels of LH. The rates of change in all these parameters between 9 and 12 months of age are greatly reduced suggesting that the changes after 9 months of age are quantitative, rather than qualitative. One parameter which does not attain a mature level of activity by 9 months of age is spermatogenesis but a sexually mature level is attained by 11 months of age. This delay may merely reflect the time required for the necessary transitions from Type A spermatogonia to terminal stage spermatids.

SUMMARY AND CONCLUSIONS

A total of 65 Holstein bulls were killed in groups of five at monthly intervals from birth to 12 months of age. The pituitary potencies and contents of LH, FSH and GH, blood plasma levels of LH, and the levels of hypothalamic LH-RF activity were assayed and changes in these parameters compared with other measurements of reproductive development from the reproductive tract.

The linear regression equation which described the increase in body weight was $\hat{Y} = 22.5 + 26.5X$ ($\hat{Y}$ = body weight in kg; X = age in months). Changes in body weight gains could not be related to reproductive development. The weight of the anterior pituitary increased linearly from 0.39 g at birth to 1.27 g at 12 months of age. There was a significant deviation from linearity at 7 and 8 months of age when anterior pituitary weight declined.

The potency of pituitary LH was least at birth and greatest at one month of age (0.76 and 4.88 ug NIH-LH-B3 equivalent per mg fresh pituitary, respectively). From 1 month of age, the potency declined irregularly with increasing age. In contrast the pituitary content of LH increased irregularly from birth to 6 months, showed a sharp decline to 8 months and then regained and maintained the level attained at 6 months. The changes in both

pituitary LH potency and content may have been associated with reproductive development but the limits of the period of puberty could not be defined by any dramatic changes in either LH parameter.

The amount of LH in the blood plasma did not change from birth to 2 months, increased to 4 months and increased again between 6 and 10 months of age. Calculations showed that the amounts of pituitary LH released per animal per day were 52, 208, 399 and 610 ug NIH-LH-B3 equivalents at 2, 4, 8 and 10 months of age, respectively. Although LH-RF activity could not be detected in hypothalami obtained from bulls of 4 months of age or less, the increase in plasma LH between 6 and 10 months of age was associated with an increase in the levels of hypothalamic LH-RF.

The pituitary potency of FSH was greatest at 2 months of age (0.24 ug NIH-FSH-S3 equivalents per mg fresh pituitary) and then declined irregularly to 11 months of age (0.07 ug per mg). However, the pituitary content of FSH, like LH, was greatest at 5 and 6 months of age. The pituitary LH:FSH ratio showed little variation between 3 and 8 months of age (avg. 14.7).

The pituitary potency of GH increased dramatically from 1 to 4 months of age (16 and 127 ug NIH-GH-B9 equivalents per mg fresh pituitary, respectively) and then declined to 24 ug equivalents per mg at 12 months of age. The pituitary content of GH followed very similar

trends. Although changes in pituitary GH levels did not appear to be related to either reproductive development or body-weight gains, the trends were very similar to the age changes in the weight of the thymus gland.

Testis weight increased from 2.47 g at birth to 203.56 g at 12 months of age. These weight changes followed a quadratic growth curve from birth to 9 months of age. Thereafter, the rate of increase declined. Changes in testicular volume showed similar trends. The testicular midpoint diameter and pole-to-pole length both increased linearly from birth to 9 months of age. Subsequent increases to 12 months of age were small. Nine months appeared to be the age at which the rate of change in all four of these parameters declined, indicating that the period of most rapid development as measured by these parameters had ended.

The amounts of RNA and DNA per testis increased rapidly from 3 months of age with the proportional increase in RNA being greater. Whereas DNA concentration declined at a diminishing rate from 7.18 mg per g at birth to 3.27 mg per g at 10 months of age, RNA concentration did not change greatly, varying from 6.13 mg per g at 4 months of age to 4.34 mg per g at 10 months of age.

The diameter of the seminiferous tubules increased linearly from birth to 10 months of age at an average rate of 16.9 microns per month. Mature spermatids were observed in histological sections from the testis of one

6 month-old bull, two 7 month-old bulls and all bulls which were 8 months of age or older. However, terminal stage spermatids (Stages VI to VIII) and/or sperm were detected in testicular homogenates from one 5 month, two 6 month, three 7 month and all bulls of 8 months of age or older. The concentration of testicular sperm increased from 4.24 million per gram testicular parenchyma at 5 months to 52.83 million per gram at 11 months of age. Thus, spermatogenesis per gram of testis in an 11 month old Holstein bull is similar to that previously reported for mature bulls. Changes in sperm production from 11 months of age are primarily due to increases in testicular size.

The weight of the epididymis increased with advancing age, similarly to testicular weight, with the exception that the epididymal curve was continuous to 12 months of age. This increase in weight was largely due to weight changes in the caput epididymidis which showed a 40-fold increase from birth to 12 months of age. Comparable proportional weight changes is the corpus and cauda epididymides were 20-fold and 26-fold increases, respectively. The development of the epididymal epithelium commenced in the cauda epididymidis and progressed towards the testis. Only one of the five bulls aged 6 months had sperm in all segments of the excurrent ducts but from 8 months of age, all bulls had sperm in

all segments but the variation in sperm numbers in any one segment within an age group was large.

The changes in the weight and DNA and RNA contents of the paired seminal vesicles showed trends which were similar to each other and to changes in the levels of plasma LH. That is, these seminal vesicular parameters showed increases from 2 to 4 months, little change to 6 months followed by dramatic increases to 9 months of age. The increases in RNA were greater than those for DNA from 1 to 4 months of age, but thereafter proportional changes in the nucleic acids were similar. The fructose and citric acid contents of the paired seminal vesicles both increased dramatically from 6 to 9 months of age. The fructose content of 101.9 mg per pair of seminal vesicles at 9 months of age was greater than at any other age to 12 months, but changes in the secretory activity of the seminal vesicles was not reflected by changes in the cell height of the secretory epithelium.

The length of the penis from the tip of the glans penis to the point of attachment to the pelvis, with the sigmoid flexure extended increased linearly from 30.7 cm at birth to 80.0 cm at 9 months of age. Increases in length from 9 months of age were small. The erratic changes in the weight of the thyroid and the acini epithelial cell height did not appear to be related to reproductive development. The weight of the paired adrenal

glands increased linearly from 2.94 g at birth to 13.12 g at 10 months of age. Subsequent changes were small. The increases in the width of the zona glomerulosa and the combined width of the zonas reticularis and fasiculata from birth to 12 months of age were erratic and did not appear to be associated with reproductive development.

Puberty was defined as "the phase of bodily development during which the gonads secrete hormones in amounts sufficient to cause accelerated growth of the genital organs and the appearance of secondary sexual characters." In the Holstein bull this phase commenced at 2 months of age and was completed by 9 months of age. The development was not continuous but comprised two periods of accelerated development from 2 to 4 months and from 6 to 9 months of age, respectively. These periods were associated with increasing levels of plasma LH. Development to 6 months of age appeared to be the result of FSH stimulation in conjunction with minimal levels of LH. The greater development from 6 months of age was primarily due to increasing levels of LH which stimulated testicular androgen synthesis which, in turn, promoted seminal vesicular growth and secretion.

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APPENDICES

APPENDIX I

PARAMETERS OF SIZE OF THE REPRODUCTIVE TRACT, PITUITARY, AND BODY WEIGHT FOR INDIVIDUAL BULLS

Age	Bull No.	Testis				Epididymis			
		Average Weight	Volume[b]	Length[b]	Diameter[b]	Caput[a]	Corpus[a]	Cauda[a]	Total
		g	ml	cm	cm	g			
Birth	1	1.63	4.3	2.4	1.1	0.20	0.09	0.13	0.42
	2	3.10	4.9	2.6	1.4	0.19	0.10	0.14	0.43
	3	3.39	6.5	2.9	1.4	0.27	0.17	0.24	0.68
	4	1.69	6.3	2.2	1.3	0.24	0.12	0.16	0.49
	5	2.53	4.5	2.7	1.1	0.30	0.12	0.20	0.67
	Mean ± SE	2.47 ± 0.36	5.3 ± 0.5	2.6 ± 0.1	1.3 ± 0.1	0.24 ± 0.02	0.12 ± 0.01	0.17 ± 0.02	0.54 ± 0.06
1 Month	180	3.97	5.0	2.7	1.3	0.25	0.12	0.19	0.53
	183	2.97	4.1	3.9	1.4	0.17	0.10	0.17	0.44
	179	5.06	5.3	3.0	1.6	0.30	0.10	0.22	0.62
	182	2.80	4.4	2.9	1.6	0.23	0.11	0.18	0.55
	184	3.43	5.0	2.7	1.3	0.25	0.13	0.20	0.59
	Mean ± SE	3.47 ± 0.40	4.8 ± 0.1	2.8 ± 0.1	1.4 ± 0.1	0.24 ± 0.01	0.11 ± 0.01	0.19 ± 0.01	0.55 ± 0.02
3 Months	175	6.18	6.8	3.4	1.6	0.39	0.28	0.39	1.06
	176	9.73	11.0	4.6	2.1	0.69	0.26	0.76	1.81
	177	7.41	7.7	3.8	2.3	0.48	0.19	0.49	1.06
	178	8.87	8.3	3.8	2.2	0.56	0.20	0.46	1.34
	185	7.29	9.4	3.6	1.8	0.61	0.11	0.68	1.50
	Mean ± SE	7.68 ± 0.58	8.4 ± 0.7	3.7 ± 0.1	1.8 ± 0.1	0.54 ± 0.06	0.18 ± 0.04	0.56 ± 0.09	1.36 ± 0.16
6 Months	164	13.69	12.5	4.4	2.4	0.44	0.38	0.48	1.48
	165	15.62	14.5	4.5	2.5	0.61	0.36	0.54	1.54
	166	16.41	16.0	4.8	2.7	0.70	0.39	0.70	1.89
	167	15.84	14.5	4.4	2.1	0.71	0.34	0.69	1.61
	168	11.58	15.2	4.4	2.4	0.90	0.34	0.93	2.27
	Mean ± SE	18.38 ± 1.15	14.1 ± 1.0	4.5 ± 0.1	2.4 ± 0.1	0.67 ± 0.07	0.30 ± 0.01	0.69 ± 0.06	1.80 ± 0.23
9 Months	169	21.96	21.7	5.1	2.7	1.15	0.69	0.77	2.55
	170	23.47	24.5	5.5	2.7	1.75	0.70	1.13	3.58
	171	26.07	24.5	5.3	2.9	1.33	0.43	1.33	3.09
	172	25.6	24.0	5.6	3.0	1.48	0.69	1.37	3.54
	174	23.7	24.5	5.3	3.0	1.27	0.56	1.21	3.04
	Mean ± SE	23.87 ± 1.34	23.4 ± 1.2	5.6 ± 0.3	2.8 ± 0.1	1.39 ± 0.08	0.64 ± 0.06	1.09 ± 0.13	3.06 ± 0.14
5 Months	156	42.10	37.5	7.0	3.4	1.89	0.74	1.65	4.24
	158	35.98	34.5	6.2	3.6	1.66	0.39	1.25	3.60
	160	38.98	33.0	6.8	3.4	1.29	0.62	1.46	3.20
	161	38.48	36.0	6.6	3.2	1.36	0.80	1.55	4.08
	162	41.98	36.2	6.1	3.2	1.22	0.44	1.35	4.47
	Mean ± SE	34.16 ± 2.61	32.2 ± 1.9	5.9 ± 0.3	3.2 ± 0.1	1.29 ± 0.16	0.52 ± 0.09	1.45 ± 0.10	3.80 ± 0.29
6 Months	150	55.60	56.6	7.0	3.8	2.90	0.82	2.11	6.21
	153	23.79	18.5	5.6	3.2	1.60	0.80	1.93	3.92
	154	49.96	45.0	6.1	4.0	1.28	1.48	1.10	4.09
	155	43.62	47.0	6.7	3.5	2.46	0.81	1.35	5.08
	157	58.77	57.2	7.2	3.8	2.80	1.19	1.75	5.50
	Mean ± SE	47.21 ± 5.30	44.9 ± 7.0	6.5 ± 0.3	3.7 ± 0.1	2.21 ± 0.32	1.02 ± 0.14	1.65 ± 0.19	4.96 ± 0.43

Age	Bull No.	Testis				Epididymis			
		Average Weight	Volume[b]	Length[b]	Diameter[b]	Caput[a]	Corpus[a]	Cauda[a]	Total
		g	ml	cm	cm	g			
7 Months	143	63.18	66.5	7.2	4.2	2.79	1.39	2.21	6.32
	144	57.72	57.6	7.2	3.8	1.84	0.90	1.50	4.33
	145	85.10	78.6	7.2	4.2	3.90	1.70	1.51	6.11
	146	74.40	101.0	7.2	4.2	2.71	1.08	1.35	5.14
	147	41.30	38.5	5.9	3.8	1.71	0.69	1.50	3.89
	Mean ± SE	64.34 ± 7.40	79.2 ± 21.3	6.9 ± 0.3	4.0 ± 0.1	2.57 ± 0.38	1.14 ± 0.19	1.69 ± 0.16	5.17 ± 0.49
8 Months	137	149.46	115.0	9.3	5.8	4.49	2.00	2.60	9.39
	138	85.61	74.4	7.7	4.3	3.34	2.00	2.52	8.56
	136	136.20	132.2	9.3	5.1	5.23	1.40	2.93	9.62
	142	87.30	91.0	7.5	4.6	2.78	1.09	2.56	6.83
	148	56.30	52.5	7.2	4.0	1.62	1.44	1.61	5.10
	Mean ± SE	102.97 ± 17.3	93.0 ± 14.2	8.3 ± 0.4	4.8 ± 0.3	3.53 ± 0.65	1.68 ± 0.20	2.44 ± 0.22	7.89 ± 0.85
9 Months	120	157.3	147.0	9.3	5.6	6.11	1.05	3.52	10.63
	121	164.7	168.4	10.7	5.8	7.35	1.60	3.99	13.13
	122	149.5	152.2	9.3	5.6	6.00	0.77	3.44	10.48
	125	134.1	130.0	8.5	5.0	5.83	1.40	2.88	9.59
	126	222.6	210.0	11.4	5.6	6.39	1.38	4.03	12.89
	Mean ± SE	165.5 ± 15.0	161.9 ± 11.4	9.8 ± 0.5	5.5 ± 0.1	6.47 ± 0.30	1.23 ± 0.16	3.57 ± 0.21	11.35 ± 0.71
10 Months	124	116.4	123.0	9.0	5.3	5.05	2.59	3.85	10.83
	127	200.5	195.0	10.4	5.9	6.02	2.52	4.10	12.90
	123	135.2	120.0	9.0	4.3	5.67	2.08	3.32	10.92
	132	160.1	160.0	9.3	5.3	7.50	2.09	2.68	12.06
	134	172.3	158.0	9.6	5.8	6.03	2.89	3.11	12.13
	Mean ± SE	156.9 ± 15.0	151.2 ± 14.0	9.5 ± 0.3	5.5 ± 0.2	5.95 ± 0.32	2.46 ± 0.13	3.21 ± 0.35	11.77 ± 0.39
11 Months	108	178.2	175.0	10.2	5.9	9.36	1.18	5.71	16.46
	113	220.5	210.0	10.6	6.6	7.18	1.30	3.96	13.12
	118	156.6	157.0	9.4	5.8	7.91	3.54	4.34	14.91
	117	147.1	130.0	10.6	4.8	5.34	1.73	3.30	10.40
	119	161.4	149.0	10.6	5.6	7.23	2.24	3.84	14.46
	Mean ± SE	172.76 ± 13.00	164.2 ± 14.0	10.3 ± 0.2	5.7 ± 0.3	7.38 ± 0.67	1.80 ± 0.26	4.23 ± 0.41	13.87 ± 1.02
12 Months	101	120.5	113.0	9.3	4.8	7.55	2.19	4.21	12.93
	102	187.9	182.0	10.1	5.8	10.91	1.98	5.12	18.34
	104	205.0	205.0	10.6	6.2	8.18	1.89	4.70	14.35
	107	235.8	227.0	11.2	6.2	10.21	3.31	3.91	20.77
	109	268.6	258.0	11.5	6.2	11.36	3.05	4.40	19.23
	Mean ± SE	203.56 ± 25.00	197.0 ± 25.0	10.5 ± 0.4	5.8 ± 0.3	9.64 ± 0.74	2.48 ± 0.18	4.47 ± 0.21	17.12 ± 1.49

[a]Estimates derived from right half of reproductive tract.

[b]Estimates derived from left half of reproductive tract.

[c]Estimates derived from paired seminal vesicles.

Vas Deferens		Ampulla		Penis	Sem. Ves.[c]	Pituitary			Body Weight
Average Weight	Average Length	Average Weight	Average Length	Length	Weight	Anterior	Posterior	Total	
g	cm	g	cm	cm	g	g			kg
0.37	25.8	0.19	3.8	29.4	2.55	0.23	0.13	0.41	[illegible]
0.36	27.0	0.31	4.1	35.8	[illegible]	0.37	0.11	0.54	[illegible]
0.45	30.5	0.32	3.8	36.9	3.46	0.56	0.19	0.79	[illegible]
0.38	26.4	0.20	5.1	36.3	[illegible]	0.33	0.15	0.53	32.6
0.38	25.4	0.23	5.7	35.6	4.21	0.42	0.16	0.63	39.6
0.39 ± 0.01	27.0 ± 0.9	0.25 ± 0.09	4.5 ± 0.4	30.7 ± 2.3	3.37 ± 0.37	0.39 ± 0.05	0.13 ± 0.01	0.57 ± 0.05	38.9 ± [illegible]
0.54	34.3	0.44	6.7	34.5	[illegible]	0.4	0.1	[illegible]	[illegible]
0.54	28.3	0.16	1.9	36.9	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
0.58	29.8	0.18	4.4	34.0	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
0.46	28.0	0.24	[illegible]	39.7	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
0.31	31.2	0.13	[illegible]	29.4	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
0.49 ± 0.05	30.3 ± 1.1	0.25 ± 0.06	3.7 ± 0.9	31.7 ± 2.8	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
0.72	40.3	0.29	3.8	36.8	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
0.80	38.1	0.57	4.4	36.8	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
0.52	35.6	0.33	4.8	36.8	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
0.55	36.8	0.26	5.4	34.4	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
0.54	36.2	0.33	4.1	39.4	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
0.63 ± 0.06	37.4 ± 0.8	0.36 ± 0.06	4.5 ± 0.3	37.9 ± 0.8	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
0.93	37.5	0.53	5.7	39.4	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
1.08	38.8	0.86	7.0	44.5	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
0.88	36.2	0.68	6.0	44.5	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
1.04	39.7	0.77	6.4	45.6	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
1.11	43.2	1.09	6.9	48.7	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
1.01 ± 0.04	39.0 ± 1.2	0.78 ± 0.10	6.2 ± 0.2	42.9 ± 1.2	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
1.45	43.2	1.40	5.1	43.2	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
1.21	41.9	2.19	7.0	44.5	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
1.07	48.9	1.05	6.0	46.7	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
0.92	43.2	1.36	5.7	48.3	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
0.93	46.2	1.14	6.7	45.7	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
1.12 ± 0.09	44.7 ± 1.3	1.43 ± 0.20	6.1 ± 0.4	46.5 ± 0.8	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
1.16	49.6	1.86	6.4	68.6	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
1.56	50.8	1.60	5.1	63.5	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
1.67	50.2	1.70	6.4	74.9	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
1.33	49.9	1.28	5.1	64.8	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
0.97	45.8	1.54	7.6	52.1	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
1.34 ± 0.12	49.3 ± 0.9	1.60 ± 0.10	6.1 ± 0.5	64.8 ± 3.8	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
2.64	50.5	2.70	8.9	58.4	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
1.23	50.2	1.35	5.7	54.6	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
1.12	52.4	1.58	7.0	53.3	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
1.62	52.7	2.37	8.3	57.2	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
1.09	48.9	1.96	6.4	55.9	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
1.54 ± 0.29	50.9 ± 0.7	1.99 ± 0.25	7.3 ± 0.6	55.9 ± 0.9	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Vas Deferens		Ampulla		Penis	Sem. Ves.[c]	Pituitary			Body Weight
Average Weight	Average Length	Average Weight	Average Length	Length	Weight	Anterior	Posterior	Total	
g	cm	g	cm	cm	g	g	g	g	kg
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	1.17	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	44.8	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	0.85 ± [illegible]	0.32 ± 0.01	[illegible] ± [illegible]	[illegible] ± 7.9
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± 4.7
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	1.93	314.0
[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	1.83 ± 0.14	[illegible] ± 6.7
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	1.72	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	0.41	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	1.71	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	383.0
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	5.5 ± 0.3	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± 0.04	0.41 ± 0.01	1.77 ± [illegible]	341.1 ± [illegible]

APPENDIX II

PITUITARY AND PLASMA LH DATA FOR INDIVIDUAL BULLS

Age	Bull No.	Pituitary LH		Plasma LH		Plasma Pit. LH
		Potency	Content	Conc.	Content	
		µg/mg[a]	mg/pit[b]	µg/l[a]	µg/an[c]	µg/mg[d]
Birth	1	0.79	0.18			
	2	0.47	0.17	Pooled	Pooled	
	3	0.88	0.48	Sample	Sample	
	4	0.96	0.36			
	5	0.70	0.29			
	Mean ± SE	0.76 ± 0.08	0.30 ± 0.06	0.48	0.59	1.97
1 Month	180	6.50	2.73			
	183	5.03	1.56	Pooled	Pooled	
	179	4.14	1.61	Sample	Sample	
	182	3.82	1.64			
	184	4.92	2.02			
	Mean ± SE	4.88 ± 0.46	1.91 ± 0.22	0.41	0.63	0.33
2 Months	175	1.74	0.87	0.12	0.31	0.36
	176	3.62	2.43	0.27	0.72	0.30
	177	1.71	0.91	0.11	0.26	0.29
	178	1.79	0.98	0.19	0.46	0.47
	185	2.84	1.62	0.16	0.42	0.26
	Mean ± SE	2.34 ± 0.38	1.36 ± 0.30	0.17 ± 0.03	0.43 ± 0.08	0.34
3 Months	164	2.57	1.62	----	----	----
	165	3.03	2.42	0.19	0.65	0.27
	166	2.39	1.86	0.23	0.82	0.44
	167	4.62	3.23	0.31	0.99	0.31
	168	3.21	2.41	0.61	2.23	0.93
	Mean ± SE	3.16 ± 0.39	2.31 ± 0.28	0.34 ± 0.09	1.17 ± 0.36	0.49
4 Months	163	2.52	2.12	0.42	2.03	0.96
	170	2.90	2.18	0.66	3.25	1.49
	171	2.11	2.30	0.26	1.33	0.58
	172	2.59	1.68	0.11	0.52	0.31
	174	2.08	1.35	0.32	1.46	1.08
	Mean ± SE	2.44 ± 0.15	1.93 ± 0.18	0.35 ± 0.09	1.72 ± 0.45	0.88

Age	Bull No.	Pituitary LH		Plasma LH		Plasma Pit. LH
		Potency	Content	Conc.	Content	
		µg/mg[a]	mg/pit[b]	µg/l[a]	µg/an[c]	µg/mg[d]
5 Months	156	2.96	3.55	0.23	1.21	0.34
	158	3.52	2.71	0.51	2.67	0.99
	160	1.99	1.55	0.11	0.56	0.36
	161	4.25	4.12	0.43	2.25	0.55
	162	2.12	1.97	0.15	0.80	0.41
	Mean ± SE	2.97 ± 0.43	2.78 ± 0.47	0.29 ± 0.08	1.50 ± 0.41	0.53
6 Months	150	1.69	1.66	0.22	1.36	0.82
	153	1.90	1.63	0.31	1.79	1.10
	154	4.14	3.93	0.09	0.60	0.15
	155	3.83	4.40	0.43	3.00	0.68
	157	2.90	2.55	0.13	0.75	0.29
	Mean ± SE	2.89 ± 0.49	2.83 ± 0.57	0.24 ± 0.07	1.50 ± 0.43	0.61
7 Months	143	1.02	0.86	0.48	3.75	4.36
	144	3.12	2.56	0.46	3.33	1.30
	145	3.31	2.95	0.16	1.08	0.37
	146	1.60	1.36	0.12	0.82	0.60
	147	3.29	2.01	0.28	1.72	0.86
	Mean ± SE	2.47 ± 0.48	1.95 ± 0.38	0.30 ± 0.07	2.14 ± 0.59	1.50
8 Months	136	2.50	2.03	0.40	3.38	1.67
	137	2.49	2.42	0.10	0.82	0.34
	138	1.78	1.39	0.63	4.99	3.59
	142	1.42	1.22	0.61	4.83	3.96
	148	1.18	1.12	0.34	2.60	2.32
	Mean ± SE	1.87 ± 0.27	1.64 ± 0.25	0.42 ± 0.10	3.32 ± 0.77	2.38
9 Months	120	2.74	2.66	0.22	1.95	0.73
	121	2.28	2.87	0.55	4.94	1.72
	122	1.81	2.26	0.24	2.22	0.98
	125	1.53	1.97	0.23	2.13	1.08
	126	1.88	2.71	0.84	8.25	3.04
	Mean ± SE	2.05 ± 0.21	2.49 ± 0.17	0.42 ± 0.12	3.90 ± 1.22	1.51

Age	Bull No.	Pituitary LH		Plasma LH		Plasma Pit. LH
		Potency	Content	Conc.	Content	
		µg/mg[a]	mg/pit[b]	µg/l[a]	µg/an[c]	µg/mg[d]
10 Months	123	3.21	4.33	0.41	3.98	0.92
	124	2.05	2.44	0.24	2.27	0.93
	127	2.12	2.37	0.93	9.74	4.11
	132	1.38	1.45	0.69	7.25	5.00
	134	2.64	3.22	0.22	2.15	0.67
	Mean ± SE	2.28 ± 0.31	2.76 ± 0.48	0.50 ± 0.14	5.08 ± 1.48	2.33
11 Months	108	1.46	2.22	0.32	3.72	1.68
	113	2.95	4.57	0.29	3.49	0.76
	117	2.73	3.36	0.18	1.93	0.57
	118	2.30	2.16	0.75	8.33	3.86
	119	1.14	1.63	0.37	4.07	2.50
	Mean ± SE	2.12 ± 0.35	2.79 ± 0.53	0.38 ± 0.10	4.31 ± 1.07	1.87
12 Months	101	1.77	2.14	0.31	3.75	1.75
	102	2.04	2.43	0.33	3.80	1.56
	104	1.85	2.29	0.73	9.15	4.00
	107	2.16	2.96	0.24	3.22	1.09
	109	2.00	2.72	0.73	7.41	2.72
	Mean ± SE	1.96 ± 0.07	2.51 ± 0.15	0.47 ± 0.11	5.47 ± 1.18	2.22

[a] µg NIH-LH-B3 equivalent per mg fresh pituitary or per l plasma.

[b] mg NIH-LH-B3 equivalent per anterior pituitary.

[c] µg NIH-LH-B3 equivalent in total plasma (µg/l x body wt. x 0.035).

[d] Total plasma LH (µg) ÷ Total pituitary LH (mg).

APPENDIX III

BIOCHEMICAL PARAMETERS FOR TESTES AND SEMINAL VESICLES

Age	Bull No.	Testis^a						
		RNA		DNA		RNA/DNA	RNA	
		Conc.	Total	Conc.	Total		Conc.	Total
		mg/g	mg	mg/g	mg		mg/g	mg
Birth	1	4.21	4.80	6.69	7.63	0.63	2.95	8.41
	2	5.21	11.72	7.29	16.40	0.71	4.09	10.84
	3	6.75	15.09	9.20	24.66	0.73	1.36	4.71
	4	4.59	6.01	6.59	8.63	0.70	5.35	17.44
	5	4.47	7.91	6.15	10.89	0.73	4.86	20.46
	Mean ± SE	5.04 ± 0.46	9.71 ± 2.41	7.18 ± 0.55	13.64 ± 3.14	[illegible] ± 0.08	3.72 ± 0.71	12.37 ± 2.89
1 Month	179	5.73	23.95	4.55	19.02	1.26	2.59	4.62
	180	5.31	16.44	6.79	19.19	0.86	2.72	5.58
	182	4.93	11.14	6.98	15.77	0.71	2.35	5.17
	183	4.56	11.38	6.79	15.50	0.84	1.60	3.41
	184	5.78	14.16	6.45	[illegible]	0.55	2.00	3.12
	Mean ± SE	[illegible]	15.39 ± [illegible]	6.2[illegible] ± 0.4	[illegible]	0.90 ± 0.09	2.25 ± 0.20	4.38 ± 0.48
2 Months	175	6.15	29.3[illegible]	[illegible]	[illegible]	1.16	3.42	8.11
	176	6.27	[illegible]	6.40	[illegible]	0.97	4.09	16.44
	177	5.57	34.6[illegible]	4.94	[illegible]	1.13	3.32	8.17
	178	[illegible]	36.[illegible]	[illegible]	[illegible]	[illegible]	3.37	9.96
	185	6.13	[illegible].93	[illegible]	[illegible]	1.59	4.09	14.48
	Mean ± SE	5.94 ± 0.14	27.56 ± 3.90	[illegible]	[illegible]	1.11 ± 0.05	3.65 ± 0.15	11.43 ± 1.71
3 Months	164	5.[illegible]	64.57	4.[illegible]	[illegible]	1.29	4.09	12.97
	165	5.35	4[illegible].51	[illegible]	[illegible]	1.10	5.57	33.70
	166	4.[illegible]	[illegible].71	5.50	[illegible]	[illegible]	4.79	27.78
	167	5.73	45.11	[illegible]	47.5[illegible]	1.03	4.03	19.55
	168	4.34	37.06	4.37	[illegible]	0.99	4.59	45.26
	Mean ± SE	[illegible].19 ± 0.2[illegible]	49.26 ± [illegible]	4.64 ± [illegible]	[illegible]	1.13 ± 0.06	4.61 ± 0.28	27.85 ± 5.60
4 Months	169	[illegible]	[illegible]	[illegible].21	[illegible]	[illegible]	4.86	38.98
	170	[illegible]	119.50	[illegible]	[illegible]	1.54	4.47	81.30
	171	6.17	14[illegible]	[illegible]	[illegible]	1.7[illegible]	4.47	63.74
	172	6.65	99.83	4.[illegible]	[illegible]	1.32	4.86	57.83
	174	[illegible]	109.[illegible]	[illegible].01	[illegible]	1.10	5.64	58.20
	Mean ± SE	6.13 ± [illegible]	[illegible] ± 7.95	4.09 ± [illegible]	[illegible]	1.54 ± 0.14	4.86 ± 0.22	60.09 ± 6.87
5 Months	156	5.3[illegible]	184.[illegible]	[illegible]	[illegible]	1.35	3.53	63.36
	158	4.79	137.[illegible]	3.61	[illegible]	1.59	3.64	54.27
	160	5.13	145.89	[illegible]	[illegible]	1.63	4.16	60.86
	161	5.73	155.[illegible]	3.96	[illegible].76	1.45	4.27	72.57
	162	[illegible].81	131.59	5.05	[illegible]	1.14	3.75	62.63
	Mean ± SE	5.56 ± [illegible]	[illegible]7.19 ± 11.67	3.95 ± 0.3[illegible]	111.[illegible] ± 11.[illegible]	1.43 ± 0.09	3.87 ± 0.14	62.74 ± 3.24
6 Months	152	6.75	323.33	4.55	217.95	1.48	3.98	95.52
	153	5.14	134.31	3.85	100.60	1.34	4.72	59.42
	154	5.57	262.24	3.69	173.73	1.51	2.81	38.50
	155	5.07	184.45	4.19	152.43	1.21	3.42	55.40
	157	5.21	272:17	3.75	195.90	1.39	3.86	44.58
	Mean ± SE	5.55 ± 0.31	235.30 ± 33.63	4.01 ± 0.15	168.12 ± 20.11	1.39 ± 0.05	3.76 ± 0.32	58.68 ± 9.94

Age	Bull No.	Testis[a] RNA Conc.	Testis[a] RNA Total	Testis[a] DNA Conc.	Testis[a] DNA Total	Testis[a] RNA/DNA	Seminal vesicles[b] RNA Conc.	Seminal vesicles[b] RNA Total
		mg/g	mg	mg/g	mg		mg/g	mg
7 Months	143	5.81	318.66	3.48	188.14	1.69	3.13	76.04
	144	4.93	329.54	3.[illegible]	174.[illegible]	1.91	[illegible].01	87.[illegible]
	145	4.72	399.31	[illegible].[illegible]	[illegible].26	1.89	3.[illegible]	[illegible].[illegible]
	146	5.14	323.1[illegible]	3.[illegible]	[illegible].[illegible]	1.80	2.[illegible]	81.49
	147	4.09	250.1[illegible]	3.[illegible]	1[illegible].[illegible]	1.34	[illegible].[illegible]	[illegible].[illegible]
	Mean ± SE	4.94 ± 0.28	[illegible].[illegible] ± 44.71	[illegible].[illegible] ± [illegible].[illegible]	194.59 ± 26.96	1.47 ± [illegible].[illegible]	[illegible].[illegible] ± 0.27	[illegible].[illegible] ± [illegible].[illegible]
8 Months	136	3.98	496.31	[illegible].[illegible]	[illegible].67	1.43	3.[illegible]	[illegible].[illegible]
	137	4.72	[illegible].38	3.[illegible]	[illegible].17	1.[illegible]	[illegible].[illegible]	[illegible].[illegible]
	138	4.69	381.89	3.[illegible]	[illegible].[illegible]	1.[illegible]	[illegible].17	[illegible].[illegible]
	142	4.72	350.78	3.[illegible]	[illegible].[illegible]	1.[illegible]	[illegible].[illegible]	[illegible].[illegible]
	148	4.79	[illegible].92	3.[illegible]	1[illegible].72	1.32	3.[illegible]	[illegible].[illegible]
	Mean ± SE	4.56 ± 0.15	[illegible].[illegible] ± [illegible].[illegible]	[illegible].[illegible] ± [illegible].[illegible]	[illegible].[illegible] ± 47.[illegible]	1.38 ± 0.06	[illegible].[illegible] ± 0.[illegible]	[illegible].[illegible] ± [illegible].[illegible]
9 Months	120	5.14	766.39	3.[illegible]	534.14	1.44	3.64	[illegible].[illegible]
	121	3.92	[illegible].[illegible]	[illegible].[illegible]	[illegible].89	1.50	3.01	[illegible].[illegible]
	12[illegible]	[illegible].21	[illegible].[illegible]	[illegible].[illegible]	[illegible].[illegible]	[illegible].28	[illegible].81	[illegible].[illegible]
	125	3.91	[illegible].01	[illegible].[illegible]	[illegible].16	1.29	3.[illegible]	[illegible].[illegible]
	126	5.38	1,231.36	[illegible].[illegible]	[illegible].62	1.48	3.79	[illegible].41
	Mean ± SE	4.59 ± 0.39	[illegible].[illegible] ± [illegible].[illegible]	[illegible].[illegible] ± [illegible].[illegible]	[illegible].[illegible] ± [illegible].11	1.40 ± 0.05	[illegible].[illegible] ± 0.17	[illegible].[illegible] ± [illegible].[illegible]
10 Months	123	4.03	[illegible].99	3.10	[illegible].30	1.30	3.21	[illegible].[illegible]
	124	4.72	[illegible].[illegible]	[illegible].[illegible]	[illegible].[illegible]	1.38	3.81	[illegible].[illegible]
	127	4.21	[illegible].39	3.01	[illegible].19	1.40	3.42	[illegible].71
	132	4.59	[illegible].[illegible]	[illegible].[illegible]	[illegible].43	1.30	[illegible].[illegible]	[illegible].[illegible]
	134	[illegible].16	[illegible].[illegible]	3.01	[illegible].49	1.38	3.01	[illegible].31
	Mean ± SE	4.34 ± 0.13	[illegible].16 ± [illegible].[illegible]	[illegible].[illegible] ± 0.14	[illegible].40 ± [illegible].[illegible]	1.33 ± 0.03	3.13 ± 0.10	[illegible].19 ± [illegible].[illegible]
11 Months	108	4.72	[illegible].99	[illegible].96	[illegible].66	1.59	[illegible].76	[illegible].[illegible]
	113	4.09	[illegible].[illegible]	3.[illegible]	[illegible].40	1.[illegible]	4.16	[illegible].[illegible]
	117	5.14	[illegible].[illegible]	[illegible].[illegible]	[illegible].74	0.77	[illegible].[illegible]	[illegible].[illegible]
	118	4.72	[illegible].40	3.[illegible]	[illegible].30	1.41	[illegible].[illegible]	81.47
	119	4.66	[illegible].[illegible]	3.[illegible]	[illegible].64	1.30	[illegible].[illegible]	[illegible].21
	Mean ± SE	4.67 ± 0.17	[illegible].[illegible] ± [illegible].[illegible]	[illegible].[illegible] ± [illegible].[illegible]	[illegible].[illegible] ± [illegible].[illegible]	1.27 ± 0.17	3.01 ± 0.29	[illegible].[illegible] ± [illegible].[illegible]
12 Months	101	4.03	[illegible].[illegible]	3.06	[illegible].67	1.32	[illegible].[illegible]	[illegible].[illegible]
	102	4.40	[illegible].[illegible]	3.[illegible]	[illegible].24	1.23	[illegible].[illegible]	[illegible].[illegible]
	104	5.73	1,[illegible].[illegible]	[illegible].[illegible]	[illegible].[illegible]	1.40	[illegible].[illegible]	[illegible].[illegible]
	107	4.86	1,[illegible].[illegible]	[illegible].[illegible]	[illegible].13	1.30	[illegible].[illegible]	[illegible].[illegible]
	109	4.59	1,1[illegible].[illegible]	[illegible].[illegible]	[illegible].[illegible]	1.30	[illegible].[illegible]	[illegible].[illegible]
	Mean ± SE	4.72 ± 0.29	[illegible].[illegible] ± [illegible].[illegible]	[illegible].[illegible] ± 0.17	[illegible].[illegible] ± [illegible].[illegible]	1.31 ± 0.03	[illegible].[illegible] ± 0.23	[illegible].[illegible] ± [illegible].[illegible]

[a]Estimates derived for parenchymal tissue of right testis.

[b]Estimates derived for paired seminal vesicles.

Seminal Vesicles[b]						
DNA		RNA/DNA	Fructose		Citric Acid	
Conc.	Total		Conc.	Total	Conc.	Total
mg/g	mg		mg/g	mg	mg/g	mg
2.36	50.98	1.50	1.39	29.96	3.04	65.60
2.69	53.24	1.08	0.60	11.95	1.60	31.57
3.06	85.68	1.15	0.67	18.64	1.12	31.36
3.53	60.21	1.35	1.30	22.46	5.13	88.57
2.28	38.27	1.25	0.85	15.66	1.1	18.89
2.58 ± 0.14	55.68 ± 9.21	1.27 ± 0.08	0.93 ± 0.17	20.74 ± 4.55	2.12 ± 0.53	46.26 ± 13.55
3.74	54.88	1.21	1.57	30.43	1.50	30.04
2.28	50.84	1.11	1.39	30.93	2.75	61.37
1.72	44.72	1.26	1.55	40.35	1.50	39.00
2.04	55.08	1.47	1.83	49.38	3.23	87.13
2.66	48.41	1.40	0.84	8.04	3.10	56.42
2.29 ± 0.19	50.79 ± 1.97	1.32 ± 0.08	1.38 ± 0....	31.82 ± 6.38	2.42 ± 0.38	54.79 ± 9.88
4.28	76.85	1.60	1.59	46.47	1.36	41.96
4.46	120.28	1....	4.37	115.06	1.69	81.96
1.75	71.75	1.51	2....	103.61	2....	89.42
4.51	114.53	1....	3.09	91....	4....	101.32
1.65	66.90	2.07	4.15	153.5	1.74	65.88
3.15 ± 0.19	87.49 ± 1....	1.60 ± 0.13	2.53 ± 0....	120.36 ± 17....	1.59 ± 0.17	76.11 ± 10.29
4.74	96.17	1.17	1.81	63.67	1.41	49.32
3.51	75.77	1.22	1.63	35.81	2.86	97.59
5.15	99.47	1.61	2....	112.9.	2.4.	112.97
4.44	83.20	1.32	2.67	49.72	2.75	93.84
2.44	75.64	1....	1.53	42.75	1.79	55.33
3.41 ± 0.10	86.65 ± 4.73	1.... ± 0.03	1.93 ± 0....	59.... ± 15.0.	2.25 ± 0....	91.81 ± 12.49
1.55	55.84	1....	2.1.	98.67	2....	101.39
2....	86.5.	1....	2.06	97.56	1.33	91.18
2....	101.3.	2....	1.91	10....	1.6.	93.12
2....	83....	2....	3....	144.41	4.71	151.69
2.01	77....	2....	2.06	77.56	2.75	106.78
1.97 ± 0.69	80.08 ± 5.6.	1.... ± 0.21	2.3. ± 0....	93.52 ± 5....	2.... ± 0.55	108.83 ± 14.92
2.61	40.55	1.5.	0.67	12.08	0.9.	16.96
2.78	41....	1.5.	0....	7.3.	1.07	15.98
2.44	3..70	1.79	0.42	6.1.	1.03	15.00
2.96	56.3.	1.44	0.49	8.38	1.2.	20.65
3.15	5..61	1.19	0.63	10.50	0.85	14.19
2.79 ± 0.13	45.79 ± 3.42	1.45 ± 0.09	0.54 ± 0.05	8.39 ± 1.07	1.02 ± 0.06	16.56 ± 1.12
2.13	51.12	1.37	1.02	24.48	1.79	42.84
3.15	39.66	1.50	0.76	9.62	0.68	8.51
2.36	32.33	1.19	0.78	10.70	1.50	20.55
2.78	46.04	1.23	0.78	12.65	0.52	8.39
3.01	34.77	1.28	0.58	6.65	0.44	5.07
2.67 ± 0.19	40.58 ± 3....	1.41 ± 0.13	0.78 ± 0.07	12.80 ± 3.08	0.98 ± 0.28	17.07 ± 6.96

Seminal Vesicles[b]						
DNA		RNA/DNA	Fructose		Citric acid	
Conc.	Total		Conc.	Total	Conc.	Total
mg/g	mg		mg/g	mg	mg/g	mg
2.87	8.18	1.63	0.07	[illegible]	[illegible]	[illegible]
3.49	9.25	1.17	0.06	[illegible]	[illegible]	[illegible]
4.19	14.50	0.8[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
4.19	13.66	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
3.3[illegible]	[illegible].66	1.4[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
3.6[illegible] ± 0.36	11.9[illegible] ± [illegible]	1.[illegible] ± [illegible]	0.0[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]
2.82	5.05	0.91	[illegible]	[illegible]	[illegible]	[illegible]
3.91	8.[illegible]	[illegible].[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible].54	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
2.50 ± 0.30	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]
4.43	16.80	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
3.43	13.79	1.[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
3.53 ± 0.28	10.[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]
2.87	9.10	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
3.86	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible] ± 0.17	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible] ± 0.14	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]
2.13	150.23	1.30	[illegible]	181.40	8.48	[illegible]
2.96	131.81	1.16	[illegible]	[illegible]	[illegible]	[illegible]
1.72	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
2.63	161.43	1.40	[illegible]	[illegible]	[illegible]	[illegible]
2.28	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
2.32 ± 0.21	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]

APPENDIX IV

GONADAL AND EXTRA-GONADAL SPERM NUMBERS FOR INDIVIDUAL BULLS

Age	Bull No.	Gonadal Sperm[a]		Epididymal Sperm				Vas. Def.	Ampulla
		Conc.	Total	Caput	Corpus	Cauda	Total		
		$x10^6$/g	$x10^9$	---------- $x10^9$ ----------				$x10^7$	$x10^7$
5 Months	156	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
6 Months	155	6.80	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	157	6.80	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	Mean	6.80	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
7 Months	146	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	145	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	147	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	Mean	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
8 Months	138	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	137	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	136	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	135	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	140	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	Mean ± SE	37.87 ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]
9 Months	121	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	120	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	125	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	126	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	122	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	Mean ± SE	50.87 ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	1.34 ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]
10 Months	134	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	127	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	132	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	133	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	124	43.24	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	Mean ± SE	38.99 ± 1.77	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	1.84 ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]
11 Months	106	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	143	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	118	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	117	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	119	51.76	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	Mean ± SE	52.83 ± 4.19	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]
12 Months	107	46.23	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	102	50.82	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	101	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	104	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	109	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
	Mean ± SE	57.33 ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]	[illegible] ± [illegible]

[a]Estimates derived from right half of reproductive tract.

APPENDIX V

HISTOLOGICAL DATA FROM THE REPRODUCTIVE TRACTS OF INDIVIDUAL BULLS

Age	Bull No.	Testis		Caput[a]		Corpus[a]
		Tub. Diam.[b]	Development[c]	Cell Height[d]	Tub. Diam.[e]	Cell Height
		microns		----------	microns	----------
Birth	1	47.6	A	6.1	60.4	18.9
	2	43.3	A	17.7	61.0	14.0
	3	39.7	A	26.2	100.7	23.8
	4	42.1	A	12.8	81.1	15.3
	5	41.5	A	6.7	110.4	17.1
	Mean ± SE	42.8 ± 1.3		13.9 ± 3.7	103.1 ± 10.2	17.8 ± 1.7
1 Month	179	48.8	A	18.3	80.5	20.1
	180	46.8	A	17.1	55.5	8.5
	182	42.1	A	14.6	99.4	20.1
	183	42.1	A	15.3	97.6	17.1
	184	48.8	A	24.4	72.0	24.4
	Mean ± SE	45.5 ± 1.5		17.9 ± 1.7	81.0 ± 8.2	18.0 ± 2.7
2 Months	175	56.7	A	23.2	76.3	32.3
	176	62.8	A	29.9	125.1	42.7
	177	53.1	A	14.6	117.7	27.5
	178	56.6	A	34.3	116.9	15.3
	186	56.7	A	21.4	136.8	39.7
	Mean ± SE	57.9 ± 1.3		24.7 ± 3.4	108.5 ± 8.5	31.5 ± 4.8
3 Months	164	73.8	B	29.9	131.8	36.0
	165	62.5	A	15.9	131.3	54.9
	166	70.2	A	30.5	112.2	32.9
	167	56.7	A	20.1	85.4	40.3
	168	69.5	A	36.6	127.5	49.4
	Mean ± SE	65.1 ± 3.8		26.6 ± 3.8	137.7 ± 28.0	42.7 ± 4.1
4 Months	163	81.7	B	32.3	133.6	29.9
	170	90.3	A	32.9	145.2	42.7
	171	79.3	B	34.2	142.1	54.3
	172	91.5	B	42.7	203.1	54.3
	174	70.2	A	29.9	137.3	33.6
	Mean ± SE	82.6 ± 3.9		34.4 ± 2.2	152.3 ± 12.9	43.0 ± 5.1
5 Months	156	102.5	B	36.6	123.8	58.0
	158	95.2	B	37.2	149.5	55.5
	160	70.2	B	63.4	210.5	36.0
	161	109.2	B	32.9	119.0	41.5
	162	104.3	B	47.6	231.8	48.8
	Mean ± SE	96.3 ± 6.9		43.5 ± 5.5	166.9 ± 23.0	48.0 ± 4.2
6 Months	150	183.0	C	49.4	195.8	65.9
	153	89.1	B	20.1	115.9	68.9
	154	124.4	B	42.7	154.3	60.4
	155	118.3	B	42.1	164.7	32.9
	157	136.0	B	44.6	136.0	48.2
	Mean ± SE	130.2 ± 4.8		40.0 ± 5.1	153.3 ± 13.5	55.3 ± 6.6

Corpus[a]		Cauda[a]		Vas Deferens		Ampulla	Sem. Ves.
Tub. Diam.	Cell Height	Tub. Diam.	Cell Height	Tub. Diam.	Cell Height	Cell Height	Cell Height
---------- microns ----------							
7[illegible].3	[illegible].8	[illegible].4	18.2	[illegible]6	26.9	18.3
7[illegible].3	[illegible].1	[illegible].[illegible]	16.3	[illegible]8	19.8	24.4
9[illegible].[illegible]	[illegible].1	[illegible].1	16.8	1[illegible].8	16.8	24.4
7[illegible].9	[illegible].8	[illegible].[illegible]	18.3	1[illegible].3	22.2	20.4
7[illegible].6	1[illegible].[illegible]	[illegible]6.0	16.8	1[illegible].6	16.8	30.6
78.8 ± 4.6	[illegible].[illegible] ± [illegible].7	[illegible].1 ± 16.8	15.9 ± 1.0	1[illegible].4 ± 21	20.3 ± 1.7	23.6 ± 2.1
84.8	[illegible].6	[illegible].9	27.6	1[illegible].3	18.3	15.3
70.2	[illegible].3	1[illegible].7	21.4	[illegible]2	10.7	13.7
68.9	[illegible].3	[illegible].4	24.4	[illegible]4	21.4	18.3
7[illegible].3	1[illegible].7	[illegible].[illegible]	[illegible].9	1[illegible].[illegible]	15.3	18.3
[illegible]3.3	[illegible].1	[illegible].[illegible]	18.3	[illegible]44	16.8	10.7
78.7 ± 4.4	[illegible].[illegible] ± [illegible].2	1[illegible].[illegible] ± 10.[illegible]	[illegible].[illegible] ± 1.[illegible]	1[illegible].9 ± 15	16.5 ± 1.8	15.3 ± 1.4
110.4	[illegible].3	[illegible].[illegible]	[illegible].4	1[illegible]	26.9	16.8
1[illegible].4	[illegible].4	[illegible].[illegible]	[illegible].[illegible]	[illegible].[illegible]	18.3	21.4
118.9	[illegible].6	1[illegible].[illegible]	[illegible].9	1[illegible]	[illegible].9	26.0
134.8	[illegible].4	[illegible].[illegible]	36.1	1[illegible]	13.7	21.4
1[illegible].8	[illegible].[illegible]	[illegible].[illegible]	18.3	1[illegible]	21.4	26.0
127.1 ± 6.5	[illegible].1 ± [illegible].0	[illegible].[illegible] ± [illegible].[illegible]	[illegible].[illegible] ± [illegible].4	170 ± 17	[illegible].[illegible] ± [illegible].1	[illegible].3 ± 1.7
1[illegible].8	[illegible].1	[illegible].1	[illegible].3	[illegible]05	[illegible].4	[illegible].4
[illegible].9	[illegible].[illegible]	[illegible].[illegible]	[illegible].7	[illegible]09	[illegible].4	19.8
119.0	[illegible].[illegible]	[illegible].[illegible]	[illegible].9	[illegible]48	18.3	[illegible].4
[illegible].4	[illegible].[illegible]	[illegible].1	[illegible].[illegible]	[illegible]78	18.3	26.0
[illegible].9	[illegible].[illegible]	1[illegible].[illegible]	30.[illegible]	[illegible]06	[illegible].4	[illegible].[illegible]
158.0 ± 16.[illegible]	[illegible].[illegible] ± [illegible].[illegible]	1[illegible].[illegible] ± 1[illegible].[illegible]	[illegible].[illegible] ± [illegible].[illegible]	[illegible].[illegible] ± [illegible]	[illegible].4 ± 1.4	[illegible].[illegible] ± 1.[illegible]
11[illegible].[illegible]	[illegible].[illegible]	[illegible].[illegible]	[illegible].[illegible]	[illegible].[illegible]	16.8	[illegible].[illegible]
1[illegible].[illegible]	[illegible].[illegible]	[illegible].[illegible]	[illegible].4	[illegible].[illegible]	19.3	[illegible].4
[illegible].[illegible]	[illegible].[illegible]	1[illegible].[illegible]	[illegible].[illegible]	[illegible].[illegible]	[illegible].4	1[illegible].[illegible]
[illegible].1	[illegible].[illegible]	[illegible].[illegible]	[illegible].[illegible]	[illegible].[illegible]	13.7	[illegible].4
1[illegible].[illegible]	[illegible].[illegible]	[illegible].1	[illegible].[illegible]	[illegible].[illegible]	[illegible].[illegible]	[illegible].[illegible]
17[illegible].[illegible] ± [illegible].1	[illegible].[illegible] ± [illegible].[illegible]	[illegible].[illegible] ± [illegible].[illegible]	[illegible].[illegible] ± [illegible].[illegible]	[illegible].[illegible] ± [illegible]	[illegible].[illegible] ± [illegible].[illegible]	[illegible].[illegible] ± 1.[illegible]
[illegible]08.0	[illegible].[illegible]	[illegible]19.[illegible]	[illegible].[illegible]	[illegible].[illegible]	24.4	[illegible].[illegible]
231.8	[illegible].[illegible]	[illegible]31.[illegible]	[illegible].[illegible]	[illegible].[illegible]	[illegible].[illegible]	[illegible].4
143.4	[illegible].[illegible]	[illegible].[illegible]	[illegible].4	[illegible].[illegible]	21.4	[illegible].4
172.6	[illegible].[illegible]	[illegible].[illegible]	[illegible].[illegible]	[illegible].[illegible]	[illegible].[illegible]	[illegible].[illegible]
[illegible]09.2	[illegible].[illegible]	[illegible].[illegible]	[illegible].[illegible]	[illegible].[illegible]	[illegible].[illegible]	[illegible].[illegible]
193.0 ± 15.6	[illegible].[illegible] ± [illegible].[illegible]	[illegible].[illegible] ± [illegible].[illegible]	[illegible].[illegible] ± [illegible].[illegible]	[illegible].[illegible] ± [illegible]	[illegible].[illegible] ± [illegible].[illegible]	[illegible].[illegible] ± [illegible].[illegible]
[illegible]01.3	[illegible].[illegible]	[illegible].[illegible]	[illegible].[illegible]	[illegible].[illegible]	[illegible].[illegible]	[illegible].[illegible]
[illegible]15.3	[illegible].[illegible]	[illegible].[illegible]	[illegible].[illegible]	[illegible].[illegible]	[illegible].[illegible]	[illegible].[illegible]
[illegible]23.1	[illegible].[illegible]	[illegible].[illegible]	[illegible].[illegible]	[illegible].[illegible]	[illegible].[illegible]	[illegible].[illegible]
[illegible]12.2	[illegible].[illegible]	[illegible].[illegible]	[illegible].[illegible]	[illegible].[illegible]	[illegible].[illegible]	[illegible].[illegible]
[illegible]01.3	[illegible].[illegible]	[illegible].[illegible]	[illegible].[illegible]	[illegible].[illegible]	[illegible].[illegible]	[illegible].[illegible]
191.6 ± 20.5	[illegible].[illegible] ± [illegible].[illegible]	[illegible].[illegible] ± [illegible].[illegible]	[illegible].[illegible] ± [illegible].[illegible]	[illegible].[illegible] ± [illegible]	[illegible].[illegible] ± [illegible].[illegible]	[illegible].[illegible] ± [illegible].[illegible]

Age	Bull No.	Testis		Caput[a]		Corpus[a]
		Tub. Diam.[b]	Development[c]	Cell Height	Tub. Diam.[e]	Cell Height
		microns		microns		
7 Months	143	146.4	B	47.6	189.7	48.8
	144	113.5	B	42.1	166.5	47.6
	145	156.6	C	26.8	133.0	64.7
	146	126.9	C	50.6	244.6	68.9
	147	100.7	B	31.1	139.1	61.6
	Mean ± SE	128.6 ± 10.1		39.6 ± 4.6	174.6 ± 20.2	58.3 ± 4.3
8 Months	136	169.6	C	61.6	209.8	42.1
	137	170.2	C	45.8	273.3	29.9
	138	175.7	C	58.5	266.4	68.3
	142	169.6	C	38.4	291.4	67.7
	148	103.1	C	61.9	384.3	39.7
	Mean ± SE	157.6 ± 13.7		51.9 ± 4.4	274.6 ± 29.3	49.5 ± 7.8
9 Months	120	183.0	C	63.4	303.2	73.8
	121	214.1	C	38.4	198.3	44.5
	122	176.3	C	45.8	227.5	39.0
	125	139.1	C	36.6	320.3	73.2
	126	197.6	C	56.6	265.4	56.7
	Mean ± SE	131.9 ± 12.6		47.0 ± 4.8	262.9 ± 22.8	57.4 ± 7.2
10 Months	123	213.4	C	59.6	312.9	58.6
	124	155.6	C	46.4	183.0	91.9
	127	201.9	C	32.3	213.5	75.5
	132	180.6	C	81.7	369.1	48.2
	134	209.2	C	67.7	309.0	64.7
	Mean ± SE	197.2 ± 9.9		55.7 ± 8.6	276.7 ± 34.2	59.7 ± 4.8
11 Months	108	150.9	C	59.5	319.0	72.0
	113	198.3	C	32.3	284.4	79.3
	117	159.1	C	59.3	250.6	47.6
	118	132.4	C	75.9	290.4	49.4
	119	219.6	C	73.3	278.9	84.8
	Mean ± SE	193.9 ± 7.2		60.1 ± 7.9	278.1 ± 19.4	66.6 ± 7.7
12 Months	101	180.0	C	36.6	194.0	73.8
	102	196.8	C	69.5	368.1	54.9
	104	192.2	C	76.3	330.3	75.6
	107	194.0	C	88.5	372.1	45.8
	109	100.0	C	32.9	130.5	29.3
	Mean ± SE	172.4 ± 18.3		60.8 ± 11.0	286.2 ± 43.2	55.9 ± 8.7

[a]Segments of the epididymis.

[b]Diameter of the seminiferous tubules.

[c]Stage of development of the seminiferous tubules (A = All solid tubules, B = Some tubes lumenized, C = Sperm present in some tubules).

[d]Epithelial cell height of the excurrent ducts.

[e]Diameter of the excurrent ducts from the base of the secretary epithelium.

Corpus[a]		Cauda[a]		Vas. Deferens	Ampulla	Sem. Ves.
Tub. Diam.	Cell Height	Tub. Diam.	Cell Height	Tub. Diam.	Cell Height	Cell Height
----------	----------	----------	microns	----------	----------	----------
295.2	70.8	211.1	39.7	318	32.1	30.5
211.7	64.1	222.7	30.5	311	26.0	27.5
215.3	70.8	350.8	27.5	262	32.1	44.3
222.0	61.0	461.8	36.6	403	24.4	26.0
200.7	40.3	323.3	24.4	305	29.0	15.3
229.0 ± 16.9	61.4 ± 5.6	313.9 ± 46.0	31.7 ± 2.8	320 ± 23	28.7 ± 1.6	28.7 ± 4.7
142.1	62.8	288.5	27.5	470	22.9	26.0
114.7	59.8	207.4	32.1	549	24.4	27.5
234.9	48.8	329.4	30.5	305	21.4	21.4
245.8	47.6	411.8	24.4	366	22.9	24.4
154.9	75.0	248.9	29.0	281	19.8	24.4
178.5 ± 26.1	58.8 ± 5.0	297.2 ± 35.1	28.7 ± 1.3	394 ± 51	22.3 ± 0.8	24.7 ± 1.0
266.6	67.7	413.0	32.1	488	24.4	36.6
434.9	73.2	308.7	36.6	378	27.5	32.1
233.6	59.8	308.1	30.5	549	26.0	24.4
245.8	67.7	520.3	35.1	427	18.3	26.0
337.3	64.1	385.5	30.5	390	26.0	33.6
305.6 ± 35.3	66.5 ± 2.2	387.1 ± 39.3	33.0 ± 1.2	446 ± 32	24.4 ± 1.6	30.5 ± 0.6
274.5	54.9	339.8	22.6	366	27.8	21.4
253.2	64.7	385.5	30.5	354	19.8	27.5
283.7	45.8	602.1	50.8	305	43.8	26.4
391.6	72.6	305.6	45.8	415	21.4	24.4
364.2	66.5	276.9	18.3	360	26.0	27.5
313.4 ± 27.1	60.9 ± 4.7	382.0 ± 57.9	33.6 ± 6.7	360 ± 18	27.8 ± 4.3	25.4 ± 1.2
378.2	76.3	579.5	45.8	427	32.1	27.5
306.8	61.6	915.0	33.6	397	22.9	33.6
337.3	81.1	305.0	27.5	366	21.4	24.4
419.1	62.8	251.9	39.7	458	30.5	22.9
280.6	64.1	355.6	30.5	488	26.4	24.4
344.4 ± 24.7	69.2 ± 4.0	481.4 ± 121.9	35.4 ± 3.3	427 ± 22	26.7 ± 2.1	26.6 ± 1.9
308.1	67.1	303.8	30.5	598	26.0	27.5
358.7	79.3	335.5	33.6	397	29.0	24.4
336.7	54.3	498.4	32.1	366	26.0	27.5
292.8	45.1	707.6	39.7	372	20.9	66.4
154.3	29.9	194.0	29.0	312	27.5	32.1
290.1 ± 35.8	55.1 ± 8.6	407.9 ± 89.4	33.0 ± 1.8	409 ± 49	25.9 ± 1.4	35.6 ± 7.8

APPENDIX VI

WEIGHTS OF THYROID, ADRENAL AND THYMUS GLANDS AND THYROIDAL AND ADRENAL HISTOLOGY

Age	Bull No.	Thyroid		Adrenal Glands			Thymus Weight
		Weight	Cell Height	Weight[a]	Z. Glom[b]	Z.F. + R.[c]	
		g	microns	g	microns	microns	g
Birth	1	13.91	8.8	3.17	169	812	62
	2	7.49	6.7	2.52	136	634	90
	3	9.40	11.4	2.69	212	715	31
	4	16.14	9.0	3.58	183	994	89
	5	7.68	9.0	2.81	160	858	35
	Mean ± SE	10.92 ± 1.74	9.0 ± 0.8	2.94 ± 0.20	172 ± 13	803 ± 31	61 ± 13
1 Month	179	35.81	9.0	3.44	133	815	89
	180	7.99	9.5	2.98	150	786	64
	182	24.42	10.1	2.64	183	994	85
	183	26.11	8.9	4.14	183	694	85
	184	13.96	8.0	3.41	136	930	69
	Mean ± SE	21.64 ± 4.80	9.1 ± 0.3	3.32 ± 0.82	157 ± 11	844 ± 53	78 ± 5
2 Months	175	12.18	8.4	4.00	190	869	165
	176	17.95	9.1	4.11	233	765	245
	177	11.66	5.8	4.45	186	840	238
	178	18.83	10.4	4.46	193	948	289
	185	16.24	6.8	5.09	169	930	320
	Mean ± SE	15.27 ± 1.40	8.1 ± 0.3	4.42 ± 0.19	194 ± 10	870 ± 33	251 ± 26
3 Months	164	17.84	8.6	5.70	172	1,170	295
	165	38.06	8.1	5.25	189	1,015	314
	166	31.77	7.5	5.85	214	930	366
	167	32.84	7.5	5.70	233	834	345
	168	11.49	7.3	6.09	233	755	360
	Mean ± SE	26.40 ± 5.01	7.8 ± 0.2	5.71 ± 0.13	208 ± 12	941 ± 72	335 ± 14
4 Months	163	10.70	6.9	7.60	238	829	285
	170	11.80	11.6	5.29	197	1,372	483
	171	12.47	9.6	9.21	186	1,062	512
	172	35.47	6.8	6.27	220	1,122	485
	174	13.75	8.1	5.92	233	1,159	378
	Mean ± SE	16.84 ± 4.70	8.6 ± 0.3	6.86 ± 0.70	215 ± 10	1,109 ± 51	429 ± 43
5 Months	156	22.35	9.6	8.92	204	934	229
	158	9.21	8.8	9.14	193	444	170
	160	7.91	6.9	8.00	176	1,112	201
	161	11.31	11.5	9.58	172	998	158
	162	12.07	11.7	8.21	194	751	637
	Mean ± SE	12.57 ± 2.68	9.7 ± 0.3	8.77 ± 0.31	188 ± 6	848 ± 117	279 ± 90
6 Months	150	21.90	8.3	8.13	193	1,601	246
	153	13.33	10.4	8.92	163	1,255	250
	154	13.78	6.7	7.28	186	1,151	161
	155	12.88	9.4	9.48	183	1,027	289
	157	8.14	11.0	8.42	200	668	250
	Mean ± SE	14.00 ± 2.20	9.2 ± 0.8	8.45 ± 0.37	185 ± 6	1,140 ± 49	239 ± 21

Age	Bull No.	Thyroid		Adrenal Glands			Thymus Weight
		Weight	Cell Height	Weight[a]	Z. Glom[b]	Z.F. + R.[c]	
		g	microns	g	microns	microns	g
7 Months	143	9.55	11.9	8.52	238	1,130	161
	144	10.34	9.1	10.01	222	1,065	174
	145	11.85	9.3	9.12	236	1,192	166
	146	11.50	8.4	8.80	190	1,222	170
	147	7.15	11.0	8.30	258	1,308	52
	Mean ± SE	10.07 ± 0.80	9.9 ± 0.7	8.95 ± 0.30	229 ± 11	1,183 ± 41	144 ± 24
8 Months	136	12.74	7.9	10.05	169	920	173
	137	11.40	8.7	9.55	207	1,043	133
	138	25.65	8.7	9.40	190	884	109
	142	13.59	10.3	10.10	228	1,141	173
	148	9.82	7.6	10.24	226	1,272	226
	Mean ± SE	14.64 ± 3.10	8.6 ± 0.5	9.87 ± 0.17	204 ± 11	1,052 ± 71	162 ± 20
9 Months	120	12.75	9.4	11.00	218	1,166	167
	121	12.64	9.4	9.00	264	1,283	190
	122	14.35	9.9	13.[illegible]	[illegible]	1,209	229
	125	15.50	9.7	11.00	172	1,184	159
	126	14.48	11.0	14.03	186	1,294	173
	Mean ± SE	13.84 ± 1.60	9.9 ± 0.3	11.02 ± 0.78	210 ± 14	1,245 ± 29	182 ± 12
10 Months	123	19.11	8.5	12.48	193	1,391	223
	124	9.36	14.2	14.00	186	980	132
	127	18.92	11.4	10.79	179	1,250	77
	132	10.87	8.6	13.12	212	1,066	204
	134	15.06	11.6	15.30	250	1,159	174
	Mean ± SE	13.60 ± 2.10	10.8 ± 1.3	12.74 ± 0.76	204 ± 13	1,169 ± 72	180 ± 27
11 Months	108	13.93	10.9	13.29	157	1,299	128
	113	17.02	9.5	13.15	218	1,080	232
	117	21.45	8.7	11.53	257	1,172	365
	118	11.10	12.9	13.33	204	969	129
	119	14.26	10.5	13.80	298	1,481	322
	Mean ± SE	15.66 ± 1.80	11.6 ± 1.4	13.08 ± 0.40	227 ± 24	1,200 ± 89	235 ± 49
12 Months	101	24.68	10.5	14.12	219	1,465	136
	102	12.77	11.1	13.82	214	1,402	232
	104	17.34	9.4	11.20	200	891	240
	107	11.14	7.1	15.55	207	1,220	145
	109	12.52	10.2	13.44	190	1,091	126
	Mean ± SE	15.70 ± 2.35	9.7 ± 0.7	13.63 ± 0.70	206 ± 5	1,218 ± 106	175 ± 25

[a]Total weight for right and left glands.

[b]Width of zona glomerulosa.

[c]Combined width of zonas fasiculata and reticularis.